A STUDY OF POSTNATAL SKELETAL DEVELOPMENT IN THE DOMESTIC FOWL (*Gallus gallus domesticus*)

TWO VOLUMES

by

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VOLUME 1

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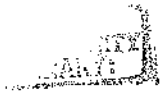

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SUMMARY

3 topics of avian postnatal skeleton development where marked differences are found in the mammalian counterpart have been studied in the domestic fowl. These are:

i. centres of ossification

ii. fusions

iii. pneumatisation

In reviewing the literature on each topic the apparent controversies encountered were examined to determine whether these involved real factual differences or only differences in terminology. As no official avian anatomical terminology has yet been agreed the terms employed in this study have been clearly defined.

The investigations were carried out on related birds from the same hatches and the period of investigation extended from the time of hatching to 182 days (26 weeks) postnatal. On some topics adult surveys were performed on a group of related birds.

i. Centres of ossification present at hatching and those developing postnatally have been identified and shown. Included were the ossification centres of the hyoid and the centres of mineralisation in the larynx, trachea, syrinx and limb tendons. The timing of appearance of the postnatally developing centres was studied and the range and mean time of appearance of each was calculated and shown. In the skull a centre which was termed the orbitosphenoid was found to develop postnatally. The extensive controversy regarding the bones in this region has been reviewed. The intervals between the initial mineralisation of the larynx and trachea as

indicated by alizarin staining and their eventual ossification identified by histological examination has been investigated. The occurrence of some variable centres in the digits of the manus, sesamoids and mineralised tendons has been surveyed in a flock of adult birds. The proximal tibial centre of the bird has been compared with the centres in this region in the mammal and their identities discussed.

ii. The sites of fusion which occur in the postnatal skeleton in the neurocranium, mandible, vertebral column, sternum, os coxae, carpus and metacarpus and tarsus have been localised and shown. The timing of each fusion has been investigated and the range of fusion time and mean fusion time for each has been calculated and shown. Where possible, comparisons have been made of the results obtained by alizarin staining with those from radiography, of male birds with female and between 2 breeds. Some aspects of the anatomy of the intercentral articulations of the 4th thoracic vertebra which may have some clinical or pathological significance have been described.

iii. The gross and histological structure of pneumatised bone has been shown. The occurrence of pneumatisation in the skull has been studied in detail in 8 birds by gross and histological examination of the individual constituent skull bones. An adult survey was performed on 51 birds to investigate the extent of pneumatisation in their skeletons and the variation present within a similar group. Comparison of male and female birds was made as far as the data permitted. Correlations were sought between the extents in different skeletal regions in individual birds. The timing of the development of the process in the postnatal skeleton has been studied and

comparisons of the rate of development in male and female birds and in 2 breeds have been made where possible. Several methods of investigation have been employed, gross examination with and without prior injection, histological examination, transillumination of the macerated skull and radiography of the humerus, and their relative merits have been discussed.

PREFACE TO STUDY

The great importance which poultry have assumed as a source of protein in the past 25 years or so with the accompanying intensification of production has resulted in a new range of disease and nutritional problems. This has stimulated considerable research activity and notable advances have been made in nutrition, immunology and pathology. Anatomical interest has also undergone a resurgence on a rather smaller scale but has not involved the skeleton to a great extent, where several basic features continue to be poorly documented.

The avian skeleton was a source of considerable interest in the late 19th century, particularly after the discovery of the fossil Archaeopteryx which provided a link with the reptilian ancestry of birds. The anatomical differences between the classes aves and mammalia resulting from their divergent courses of evolution from common reptilian precursers are in some respects very great and nowhere more than in the skeleton. This study was undertaken to investigate further 3 aspects of development in the postnatal skeleton where there are marked differences from the mammalian counterpart.

These are:-

i. Centres of ossification.

ii. Fusion between skeletal elements.

iii. Pneumatisation.

In searching the literature on these topics R.M. Strong's Bibliography of Birds was of particular value for the older literature, being a fairly comprehensive cross referenced list up to 1939. For more recent work Biological Abstracts and the Zoological Record - Aves were searched. The Index Veterinarius revealed a few items of applied

interest.

The design of the study was to follow the development of these skeletal features in a flock of closely related birds, derived from the same hatches and reared under the same conditions of management to minimise the effects of genetic, physiological and pathological factors. It was intended to study the extent of variation occurring within the groups which it was felt would be indicative of purely individual variation. Most of the investigations were performed on Golden Comet brown egg laying pullets but in a few instances it was possible to make comparison with a flock of White Leghorns which included male and female birds. The period selected for study was from hatching to 182 days (6 months) posthatching, which seemed a reasonable limit to take as the end of the "growth period" as by this age point of lay has been passed and birds are normally fully grown in terms of long bone length. On several topics surveys were performed on adult birds. These were Golden Comet birds which were the parent stock of the birds used in the growth study. They were aged $2\frac{1}{2}$ years and while they were not aged birds it seemed reasonable to consider them as representative of adults under modern conditions of management.

There is as yet no recognised standard avian anatomical nomenclature and this is a factor which has caused much confusion and difficulty in interpretation of the literature. In reporting the findings of this study it was considered essential to define clearly the terms being used. It was also the intention to illustrate the findings as clearly as possible, lack of satisfactory illustration being a conspicuous feature of some previous reports.

PART ONE

CENTRES OF OSSIFICATION IN THE POSTNATAL SKELETON

GENERAL INTRODUCTION

The chick embryo is a frequent subject for study and considerable work has been done on the embryonic aspects of skeletal development. Hamilton (1952) and Romanoff (1960) give extensive accounts of the mesenchymal, chondral and osseous stages of prenatal development and there are several reports of investigation on prenatal ossification as will be discussed subsequently. Studies on cellular aspects were initiated by Brachet (1893), extensively developed by Fell (1925), and followed into the postnatal period by Wolbach and Hegsted (1952).

Studies on the centres of ossification in the postnatal fowl skeleton are few and usually cover only limited regions. They will be discussed under appropriate headings.

There is general agreement that one major difference between the avian and mammalian skeleton is the lack of secondary ossification centres in the avian long bones (Portmann 1950, Young 1950, Bellairs and Jenkin 1960, Bradley 1960, King and McLelland 1975), though exceptions respecting the tibia and metatarsus are usually mentioned. Yet even in the face of this general unison there are 2 relatively recent texts both in fairly widespread use expressing the opposite view, namely that in birds long bones establish secondary centres,"epiphyses"(Chamberlain 1943) and that typically long bones have 3 centres but a peculiarity in birds is that the epiphyses do not appear until a day or two after hatching (McLeod et al. 1964). It is particularly difficult to give any credibility to this latter account, which goes on to say that avian long bones also have apophyses which are secondary centres in the larger projections of bones. Indeed this is not even the generally accepted definition of an apophysis but rather of a traction epiphysis (Parsons 1904-5).

This is one of many examples of confusion existing in avian anatomical literature.

In contrast to the relative lack of centres of ossification in the long bones is the peculiarity that ossification or at least calcification commonly occurs in other tissues of the body in birds. It is well known that bone plaques are found in the sclera, and other locations where the phenomenon is regularly described in the fowl, though with few details, are the cranial larynx (Bradley 1960, White 1975), tracheal rings (Bradley 1960, Bellairs 1964, King and McLelland 1975) and limb tendons (Kaupp 1916, George and Berger 1966, Evans 1969, Van den Berge 1975).

Other occasional sites have been reported and include semilunar valves (Kaupp 1918), pericardium (Ceresole 1900), and testis (Buchanan 1926).

The aims of this part of the study were to confirm the centres of ossification present at hatching and those appearing postnatally, to determine the approximate time of appearance of this latter group and to survey the incidence of variably occurring centres in sesamoids and ossified tendons in the adult.

i) SKULL

INTRODUCTION

There are several diverse sources of information on the fowl skull.

An early interest was the evolutionary aspect, an interest which has continued till the present day. The reptilian ancestry of birds was established by Gegenbaur, Huxley and others, and as the skull has particularly strong reptilian characteristics this has given rise to a number of important works on phylogeny and comparative anatomy such as

those of Huxley (1864, 1871), more recently Goodrich (1930) and the monumental work of De Beer (1937). These texts do contain many important anatomical details but they are of limited value in studying skull development in birds.

The first major anatomical account of the skull of the fowl was by Parker (1869) and is a lengthy treatise which, in addition to giving anatomical description of the skull, covers its development through a number of prenatal and postnatal stages. The detail and accuracy of this early work is considerable and amply justifies its continued use as a source of reference on the subject. It has served as the basis for several subsequent texts.

Textbooks on avian and veterinary anatomy provide very variable detail on the fowl skull. A particular difficulty lies in the tendency for the cranial sutures and synchondroses to fuse to the extent that their identity in the adult is totally obscured. Indeed this is a particular characteristic of birds especially carinates (Goodrich 1930, De Beer 1937). Illustrations of the skull in textbooks are often limited to the adult and either indicate approximate areas of bone with no attempt at precise delineation (Bradley 1960, King and McLelland 1975) or do not attempt to identify the bones separately at all (Chamberlain 1943, Feduccia 1975). They are, therefore, of no value whatsoever, in studying the separate bone elements present in the postnatal skull.

The chick has received much attention from embryologists, being a particularly suitable subject. The skull is not an exception and has been studied extensively. Thus comprehensive accounts are available up to the time of hatching (Hamilton 1952, Romanoff 1960), and Romanoff also provides some postnatal information derived from various sources.

The most significant method for studying the development of ossification in recent times has been the alizarin red S method of Dawson (1926). This has been used in several studies on the fowl skull and has provided most of the information available on ossification centres in the postnatal skeleton. The account by Schinz and Zangerl (1937) is frequently quoted but on close examination it proved disappointing in that the skull was dealt with rather more superficially than some other regions of the skeleton and the account did not include postnatal data. Furthermore, several major omissions were found notably on centres occurring around the orbit and in the mandible and hyoid. Erdmann (1940) deals only with the skull, is more comprehensive and is also one of the few sources of information on the comparative rate of ossification in the fowl, comparing Rhode Island Red with White Leghorn birds. The period of investigation is purely prenatal but centres yet to appear at hatching are listed. Fujioka's (1955) is similarly a purely prenatal account and covers the entire skeleton. On the skull the terminology is particularly aberrant. The centres present at hatching are indicated and those yet to appear are listed. Jollie (1957) gives the most comprehensive account and deals purely with the skull. The illustrations of the bones around the time of hatching are very useful in assessing the centres present at this time. The study was continued into the postnatal period but little is revealed of postnatally appearing centres. Phylogenetic significance of many aspects is discussed and there is useful reference to other avian species. The paper is much quoted in subsequent work and the illustrations reproduced with varying degrees of amendment in several texts.

The work of Sandoval (1963) is a growth study of the skull and

includes a description of its ossification closely based on Jollie (1957).

Considerable terminological variation was encountered in these various accounts of the fowl skull. However, a close study of the literature revealed a disagreement regarding the existence of skeletal elements around the orbit. This was not just a matter of terminology, though difference in terms obscured the situation even further. 3 bones were frequently described, and will for convenience be termed A, B and C. Bone A was paired and formed portions of the posterior wall of the orbit and lateral wall of the cranium. Bone B was paired and described as being near the midline and dorsal to the optic foramen, while bone C formed the bony interorbital septum. The views of various authors regarding the occurrence and identity of these bones is shown in table 1.

Author	Bone A	Bone B	Bone C
Parker (1869)	Alisphenoid	Orbitosphenoid (anterior and posterior pairs)	Ethmoid
Newton and Gadow (1896)	Alisphenoid	Orbitosphenoid	Ethmoid
Heilmann (1926)	Alisphenoid	-	Ethmoid
Goodrich (1930)	Lateral or pleurosphenoid	Orbitosphenoid	Mesethmoid
De Beer (1937)	Pleurosphenoid	Orbitosphenoid	Presphenoid or mesethmoid
Erdmann (1940)	Pleurosphenoid	Orbitosphenoid	Presphenoid
Fujioka (1955)	Pleurosphenoid	-	Orbitosphenoid
Jollie (1957)	Orbitosphenoid	-	Mesethmoid
Bellairs and Jenkin (1960)	Lateral or pleurosphenoid	Orbitosphenoid (some species)	Mesethmoid
Sandoval (1963)	Orbitosphenoid	-	Mesethmoid
Bellairs (1964)	Orbitosphenoid	-	Mesethmoid
King and McLelland (1975)	Orbitosphenoid	-	Mesethmoid

Table 1 The views of various authors concerning the existence and identity of 3 cranial bones in the domestic fowl.

Bone A was termed alisphenoid by the earlier authors but since, according to Goodrich (1930) it is not homologous with the greater wing of the mammalian sphenoid but rather represents the posterior part of the sphenethmoid of lower forms he proposed that it be termed lateral or pleurosphenoid a convention followed by de Beer (1937), Erdmann (1940), and Fujioka (1955). Bone B was identified by most of the earlier authors and termed the orbitosphenoid. Goodrich (1930) claimed this to represent the anterior part of the sphenethmoid and described it as fusing to a median ethmoid to form an extensive bony interorbital septum.

Jollie's view was rather different. He terms bone A the orbitosphenoid and describes its development from 2 centres of ossification representing the mammalian orbitosphenoid and presphenoid. He does not describe bone B at all and believes Goodrich has assumed the existence of 2 elements where only 1 is present. Sandoval (1963) follows this view though he refers to the differing opinion of Goodrich (1930). The reviews by Bellairs (1964) and King and McLelland (1975) similarly echo Jollie's interpretation. Bellairs (1964) reproduces Jollie's diagrams as do Bellairs and Jenkin (1960) but noteworthy in the latter case is the substitution of the term lateral or pleurosphenoid for Jollie's orbitosphenoid, and the mention of an additional orbitosphenoid in some birds. The orbitosphenoid was described as ossifying at 2 months of age (Parker 1869) and as appearing at an unspecified postnatal age by Erdmann (1940).

Bone C was generally termed the ethmoid by earlier authors and mesethmoid by more recent, though it was alternatively termed presphenoid by de Beer (1937) and Erdmann (1940) and even orbitosphenoid by Fujioka (1955).

The existence of an additional "presphenoid" is hinted at by several authors. Parker (1869) describes it as being the anterior of the 2 pairs of orbitosphenoids lying near the midline. Fujioka (1955) claims that it does not appear until hatching while Jollie (1957), as described above, claims that the mammalian presphenoid is incorporated into what he terms the avian orbitosphenoid and cannot, therefore, exist separately.

The epiotic and opisthotic were said by Jollie (1957) to ossify prenatally and rapidly fuse to adjacent centres. Erdmann (1940) describes them as being yet to ossify at time of hatching.

The columella or stapes was claimed to be cartilaginous at hatching by Fujioka (1955) but to be ossified at hatching by Erdmann (1940), Jollie (1957) and Romanoff (1960).

The adult turbinates were described as osseous (Sisson and Grossman 1953) and as cartilaginous (Bradley 1960). According to Fujioka (1955) their ossification has yet to occur at the time of hatching while Erdmann (1940) includes the septum internasale as a future site of ossification.

General agreement exists that the scleral ossicles have ossified by hatching (Schinz and Zangerl 1937, Nelson 1942, Fujioka 1955, Jollie 1957).

The os opticus is said to be ossified in some gallinaceous birds (Tiemeier 1950) but not to ossify in the fowl (Jollie 1957). Jollie also lists other skull centres present in some species but absent in the domestic fowl.

The mandible is described by earlier authors as being formed from 5 bones. Erdmann (1940) lists 7 by subdividing the dentary into os dentale and os mentomandibulare and additionally including the os goniale, called prearticular by Jollie (1957) and others. Jollie (1957) lists

6, dentary, splenial, angular, supraangular, articular and prearticular, though the latter 2 are considered to be fused together from their beginning. Fujioka (1955) generally agrees with this other than in terminology though he considers that in the articular only the processus angularis internus ossifies prenatally. Whether additional centres are thought to appear postnatally is not made clear. Feduccia (1975) obscures the situation by saying that generally there are 5 bones in the mandible and proceeds to list 6. He also describes a mandibular symphysis. Jollie (1957) discusses the possible occurrence of a coronoid but only quotes evidence for its existence in the golden eagle.

In general, then, there is agreement that no further mandibular centres remain to appear at time of hatching.

The situation is much less clear regarding the hyoid bones. Confusion in terminology here reaches a peak seldom equalled in vertebrate anatomy, several terms being variously applied to each element and numerous permutations of these being found among the elements. When accompanying diagrams are given, the situation can be construed but in the absence of such, a statement like that of Fujioka (1955) that only the epibranchial ossifies prenatally is totally useless. There is little point in extensively reviewing the terminology used by various authors and it seems expedient to use the nomenclature proposed by McLelland (1968) who calls the constituent elements of the paired cornua the epibranchial (proximal) and ceratobranchial (distal) and the median elements from rostral to caudal the paraglossal or entoglossal, rostral basibranchial and caudal basibranchial.

Information on ossification in the hyoid is extremely sparse. Romanoff (1960) and Erdmann (1940) both describe 1 element only ossifying

prenatally, the ceratobranchial. Jollie (1957) appears to indicate prenatal ossification only in the basal part of the epibranchial. While there is general agreement that the majority of the hyoid is ossified in the adult no precise data could be found.

MATERIALS AND METHODS

90 Golden Comet pullets were used. The first were killed within 1 hour of hatching and further birds from the same hatch were sampled at 7 day intervals to 112 days postnatal and then at 14 day intervals to 182 days postnatal. Specimens were prepared by alizarin red S staining and by radiography after prior silver nitrate impregnation. With the alizarin specimens it was found advantageous to section some skulls midsagittally for lateral examination and to prepare others by removing the roof of the cranium and disarticulating the mandible and hyoid to examine them dorsoventrally. Similarly with the radiographic specimens some were sectioned midsagittally and x-rayed laterally while the remainder were x-rayed dorsoventrally after removal of the roof of the cranium and disarticulation of mandible and hyoid.

It was found that scooping out the cranial contents in all specimens and removing the eyes in the larger alizarin specimens facilitated the penetration of alizarin and silver nitrate to the deeply located regions.

The numbers of birds subjected to these methods is summarised in table 2.

Age (Days postnatal)	METHODS				No. at each interval
	Alizarin reds		Silver nitrate radiography		
	Lat. view	D.V. view	Lat. view	D.V. view	
0	1	2	1	2	6
then at 7 day intervals till 112 days	1	1	1	1	4
then at 14 day intervals till 182 days	1	1	1	1	4

Table 2 Numbers of specimens prepared by various methods at given age intervals.

The alizarin specimens were all examined under a Zeiss operating microscope with strong transillumination and the radiographs transilluminated and examined with the aid of a hand lens.

RESULTS

A. Centres present at hatching

i. Neurocranium

Single centres were found in the single elements basioccipital, supraoccipital, mesethmoid and in the parasphenoid and basisphenoid complex in which no indication could be found of previously existing multiple centres. This element is therefore referred to subsequently as parasphenoid/basisphenoid. Single centres were also found in the paired elements exoccipital, prootic, squamosal, parietal, frontal, prefrontal and pleurosphenoid, this being the centre previously termed bone A and the application of this term will be discussed later. These centres are shown in figs. 1-5.

ii. Splanchnocranium

a) Facial and palatine skeleton

Single centres were identified for the premaxilla and vomer and for the paired maxilla, palatine, nasal, jugal, quadratojugal, pterygoid and quadrate (figs. 1-6).

b) Mandible

The various elements could not be readily identified in the radiographs due to superimposition and lack of magnification, so only the alizarin specimens were employed. Separate single centres were identified for the paired angular, supraangular,

and splenial. The articular and prearticular appeared to be fused and represented by 1 paired centre. 1 single centre was found for the dentary, with no symphysis in the midline (figs. 7, 8, 9).

c) Hyoid

Single centres were found for the paired ceratobranchial (fig. 9).

iii. Miscellaneous

Single centres were found in the columellae in all alizarin skulls examined. The columella could not be reliably identified in the radiographic specimens.

14 scleral ossicles were found bilaterally in all skulls examined.

B. Centres appearing postnatally

i. Neurocranium

A paired centre was identified at 70 days postnatal in 1 bird examined radiographically and in both examined by alizarin. It was found to occur variably in the birds aged 77 and 84 days and was present in all birds examined thereafter. It was termed the orbitosphenoid (figs. 10-12).

ii. Splanchnocranium

a) Facial and palatine skeleton

None

b) Mandible

None

c) Hyoid

Single centres for the paired epibranchial and unpaired rostral

and caudal basibranchials appeared during the course of the investigation (figs. 13-18). The entoglossal though having the appearance of a single element was found in several specimens to have a bilaterally symmetrical pair of small centres in some of the earlier specimens in which ossification was detected (fig. 16). It was presumed that these quickly coalesced into 1 single centre as found in the majority of specimens. Considerable variation was found in time of appearance of these centres as shown in tables 3 and 4.

Ages - days postnatal

	7	14	21	28	35	42	49	56	63	70	77	84	91	98	105	112	126	140	154	168	182
Entoglossal	-	-	-	-	-	-	-	-	-	-	++	-	++	+	+	+	+	+	+	+	+
	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+
Rostral	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basibranchial	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Caudal	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
Basibranchial	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
Epibranchial	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+	+	+	+	+	+
	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	+	+	+	+	+

Table 3 Occurrence of hyoid centres - alizarin

Key: - centre absent
+ centre present
++ 2 centres present

Ages - days postnatal

	7	14	21	28	35	42	49	56	63	70	77	84	91	98	105	112	126	140	154	168	182
Entoglossal	-	-	-	-	-	-	-	-	-	-	-	-	+	-	++	+	+	+	+	+	+
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+
Rostral	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basibranchial	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Caudal	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+
Basibranchial	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+
Epibranchial	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+
	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	+	+	+	+	+

Table 4 Occurrence of hyoid centres - silver nitrate radiography

Key: - centre absent
+ centre present
++ 2 centres present

The centre for the rostral basibranchial was the first to appear and was found in all birds 56 days postnatal and over. The caudal basibranchial was first seen in the alizarin birds at 70 days and in the radiographic birds at 77 days and was found in all birds over these ages.

The entoglossal was rather more variable in its time of ossification and this was first detected at 77 days in 1 alizarin specimen as a paired centre. It was of variable occurrence until 105 days but was invariably present thereafter. A single centre was first seen at 91 days (radiography) though a paired centre was subsequently found at 105 days (also by radiography). A single centre was invariably the case thereafter.

The epibranchial centre was first detected at 91 days (alizarin and radiography) and then occurred variably until 112 days after which it was always present. In all cases it was bilaterally present or absent.

iii. Miscellaneous

Only a few sutural bones associated with the frontals and parietals were noted (fig. 19).

The range of times of appearance of these postnatally appearing centres is shown in fig. 20a. The range is shown as extending from the last age where the centre is invariably absent to the first age at which it is invariably present.

The median of this range was termed the mean time of appearance. These are shown in fig. 20b.

DISCUSSION

The skull is well formed at the time of hatching being like a miniature of the adult. The multiple centres which occur initially in

many of the bones have already fused without trace giving the situation of each separate bone having 1 single centre of ossification. This would suggest that the one postnatally appearing centre in the neurocranium represents an independent element rather than an additional centre of ossification for an adjacent element.

This centre, termed orbitosphenoid, closely fits the description given by Goodrich (1930). The laterally placed centre is therefore termed pleurosphenoid. The orbitosphenoid appears very much later than all the other skull centres (except hyoid) which have all appeared by the time of hatching. This point has not been clearly emphasised by previous authors. Only Parker (1869) mentions its development at 2 months of age. Its much later time of appearance may account for Jollie (1957) failing to detect it. Although postnatal stages were included in his study the numbers and frequency of sampling were not specified. The centre is not readily recognised after its fusion to surrounding elements. Furthermore, in this work it was found that only when the eyes were first removed from the larger specimens did the alizarin dye penetrate the deeply located region where the orbitosphenoids are found. No further centres that could be called "presphenoids" were found.

The epiotic and opisthotic were not found to ossify postnatally. This is thought to confirm the view of Jollie (1957) that these fuse to other centres soon after their prenatal ossification.

Other points made by Jollie (1957) which are confirmed by this study are that the prearticular and articular are fused at hatching, that the os opticus does not ossify in the fowl and that the other centres which are listed by him as occurring in other species are absent in the fowl.

The turbinates and internasal septum did not show evidence of

ossification during the period of investigation, although this does not rule out the possibility of further ossification in this or other regions in later life.

The identity of the entoglossal or paraglossal bone has been the subject of longstanding controversy. The majority of workers have considered it to be a hyoid derivative but Kallius (1905) who was the first to apply the name paraglossal, and Crompton (1953) both consider it to be a newly evolved structure in birds not being part of the hyoid arch. De Beer and Barrington (1934) mention its origin from paired elements but the occurrence of a paired centre of ossification does not seem to have been previously reported.

Radiographic studies do not appear to have been previously employed in studying skull ossification. The silver nitrate impregnation gave sufficient contrast to identify the various centres with the exception of those of the mandible and the columella. Study of alizarin specimens with the operating microscope was useful in these cases and also particularly valuable in searching for the possible existence of other postnatally appearing centres like epiotic and opisthotic. The times of appearance of the postnatally appearing centres agreed fairly closely between the 2 techniques.

ii. VERTEBRAL COLUMN

INTRODUCTION

Several studies have been made on development of the vertebral column of the fowl, the most useful as regards ossification being the alizarin red S studies of Schinz and Zangerl (1937) Fujioka (1955) and Rumpler (1962) the latter being a little known work but providing

detailed prenatal information. Hamilton (1952) and Romanoff (1960) give useful reviews on the subject, Romanoff's being largely based on the work of Schinz and Zangerl (1937) but also containing useful comparative information from other sources. In general it can be said that certain basic principles are well known but many details remain to be elucidated.

The centra commence ossification before the neural arches, (Schinz and Zangerl 1937, Fujioka 1955, Romanoff 1960, Rumpler 1962), and the initial stages of ossification are described and shown by Schinz and Zangerl (1937).

The general direction of ossification in the vertebral column is from cranial to caudal (Hamilton 1952, Fujioka 1955, Romanoff 1960, Rumpler 1962), though Fujioka (1955) describes several exceptions in that the neural arches of atlas and axis ossify about the same time as those of cervical 11 and 13 respectively, and cervical 14 ossifies relatively late about the same time as lumbosacral 5 and that the 7th of the 10 caudal vertebrae ossifies last of all.

The typical number of centres is 3, 1 for the centrum and 2 for the arch. The atlas has 3 centres, 2 for the arch and 1 for the atlas intercentrum which is retained in place of the centrum (Romanoff 1960), while the axis in addition to its 3 basic centres has a centre for the odontoid process which is derived from the centrum of the atlas and also retains the intercentrum of the axis which becomes attached to the cranioventral face of the centrum (Schinz and Zangerl 1937, Romanoff 1960).

Costal elements are shown as separate ossification centres in the cervical vertebrae (Schinz and Zangerl 1937), and are often termed cervical ribs. Costal processes are described in the synsacral vertebrae

but are said to be absent in the lumbar region (Romanoff 1960) and prominent in the true sacrals (Schinz and Zangerl 1937). It is not made clear, however, which, if any, of these processes are represented by separate ossification centres.

Information on the timing of appearance of the centres of ossification in the centra is given by Fujioka (1955). The timing of development of the arch centres and additional centres for transverse processes and costal elements is not well known. However there is general agreement that all vertebral centres have appeared by the time of hatching.

The description of the vertebral column of birds presents considerable difficulties as the regions are not so clearly demarcated as in mammals. It is now generally accepted that the thoracic vertebrae are those which carry complete ribs i.e. with vertebral and sternal segments, the cervical vertebrae mostly having reduced costal elements. It was formerly claimed that the os notarium of the fowl was a fusion of thoracic vertebrae but by the criteria described above it must be regarded as being usually a fusion of the last cervical with the first few thoracics. The difficulties with the lumbar, sacral and coccygeal vertebrae are greater. The massive fused structure which also fuses to the paired os coxae is termed the synsacrum and the vertebrae constituting it are frequently termed synsacral, and this term is often used interchangeably with lumbosacral. However, as at least the most cranial element carries a complete rib this must be regarded as a thoracic vertebra and it is also usually claimed that one or more coccygeal vertebrae are incorporated into the mass. The identity of the remaining elements is not well understood. Bellairs and Jenkin (1960) following the account of Stresemann (1927-34) attempt to classify them on the basis of the type of transverse bars which they

carry. They consider that caudal to the true thoracic elements are several which have deep transverse projections more or less divided into dorsal and ventral bars. These they regard as thoracolumbar. Next is a sudden transition to several vertebrae having only dorsal bars which arise from their arches and fuse together. They regard these as true lumbars. These are succeeded by 2 sacral vertebrae characterised by dorsal and ventral bars joined at their tips, and finally there is a variable number of caudal elements with only single or partially divided bars.

Schinz and Zangerl (1937) give a rather different interpretation and divide the synsacrum into 3 on the basis of the site at which fusion occurs with the Ilium. They describe cranial and caudal regions with fusion achieved by the transverse processes and a central acetabular region where fusion is to the rib rudiments. They assume this to correspond to the sacral region of many reptiles which lacks transverse processes. However examination of macerated specimens showed that the acetabular region was adjacent to the area considered truly lumbar by Bellairs and Jenkin (1960). The 2 "sacral" vertebrae caudal to this do however seem to fuse via their costal processes. If this is in fact the "acetabular" region referred to by Schinz and Zangerl (1937) then they do not group the "truly lumbar" elements separately from the most cranial members of the series. Yet the lack of costal processes on these vertebrae is a striking feature of adult macerated specimens.

A further problem is variation in numbers of vertebrae between individual birds. Although this is usually quoted as being of common occurrence there are few actual reports to provide details.

In recording the ossification centres present in the vertebral column

it was intended to examine the structure of the transverse bars of the lumbosacral vertebrae to attempt to group these into regional types and also to record numbers of vertebrae present in individuals.

MATERIALS AND METHODS

3 Golden Comet pullets were killed within 1 hour of hatching and subjected to alizarin red S treatment. From the same hatch birds were sampled in groups of 3 at 7, 14 and 21 days postnatal and similarly treated. The centres of ossification in the vertebral column were examined directly and with the aid of a Zeiss operating microscope and recorded.

The intervals between the future synsacral vertebral centra appeared to be noticeably narrower than between the future free coccygeal vertebrae (figs. 21, 22). The most anterior of these wider spaces was, therefore, taken as being the joint between last future synsacral and 1st free coccygeal vertebrae. The vertebrae intervening between the last thoracic as judged by the presence of a complete rib and this 1st coccygeal were at this stage termed lumbosacral.

RESULTS

A. Centres present at hatching

i. Cervical All birds had 16 vertebrae. The centres present in each are shown in table 5.

Vertebrae	Centrum and Arch	Costal Elements	Other
C1	Arch (2)	0	Intercentrum (1)
C2	Centrum (1) Arch (2)	0	Intercentrum (1) Odontoid process (1)
C3	Centrum (1) Arch (1)	2	0
C4-5	Centrum and arch variably fused	2	0
C6-12	Centrum and arch fused	2	0
C13-14	Centrum and arch variably fused	2	0
C15-16	Centrum (1) Arch (1)	2	0

Table 5 Centres of ossification present in cervical vertebrae at time of hatching.

The centres found in the atlas are shown in fig. 23, the axis in figs. 24 and 25, cervical 13 in fig. 26a and the costal elements in fig. 26b.

ii. Thoracic All birds had 5 vertebrae. All had 1 centre for the arch and 1 for centrum. The costal elements are considered under the heading of ribs and sternum (figs. 27a and 27b).

iii. Lumbosacral All birds had 15 vertebrae. The centres present in each are shown in table 6.

Vertebrae	Centrum and Arch	Transverse Processes	Costal Elements
LS 1 - 3	Centrum (1) Arch (1)	-	-
LS 4 - 5	Centrum (1) Arch (2)	-	-
LS 6	Centrum (1) Arch (2)	2 (variably present)	-
LS 7	Centrum (1) Arch (2)	2	-
LS 8	Centrum (1) Arch (2)	2	2 (variably present)
LS 9	Centrum (1) Arch (1 or 2)	2 (variably fused together)	2
LS 10-15	1 single centre		

Table 6 Centres of ossification present in lumbosacral vertebrae at time of hatching.

The centres identified in the lumbosacral vertebrae are shown in figs. 21, 22, 28, 29, 30.

iv. Coccygeal All birds had 8 vertebrae. All consisted of 1 centre of ossification as shown in fig. 31.

B. Centres appearing postnatally

All 9 birds had 16 cervical, 5 thoracic and 15 lumbosacral. 7 had 8 coccygeals and the remaining 2 had 9.

No additional ossification centres were identified.

DISCUSSION

The only variation in numbers of vertebrae in this small sample (12 birds) was that 2 had 9 coccygeals and the remainder 8.

Separate centres for transverse processes were found only in the region lumbosacral 6-9. No bird had more than 3 pairs. The vertebrae possessing these varied slightly despite no overall variation in number. Neglecting the thoracics, separate centres for costal elements occurred at hatching in cervicals 3-16, variably in lumbosacral 8 and constantly in lumbosacral 9. Transverse and costal processes were recognisable in the more caudal members of the series. No separate ossification centres were found. Further investigation is, however, necessary to find whether mesenchymal, chondral or even osseous centres might occur here prior to hatching before the members of the series can be reliably classified on this basis. For the remainder of this study the future synsacral vertebrae other than the first will be referred to as lumbosacral and the vertebrae caudal to these as coccygeal.

The fusion of centres which was found at hatching did not entirely reflect the pattern of appearance of ossification centres described by

Hamilton (1952), Fujioka (1955) and Romanoff (1960). Cervicals 6-12 all had the centrum and arch fused whereas the lower members of the series had either variable or no fusion. However cervicals 3-5 were also found variably fused or not fused. When fusion was starting it was found to occur at the cranial extremities of centrum and arch. In lumbosacrals 4-8 2 ununited centres were found in the arch. Numbers 1-3 showed the more advanced state where these had coalesced to form 1 arch centre but below the 9th lumbosacral all vertebrae were represented by 1 single centre only. Again further investigation is necessary in the prenatal and neonatal periods to detail the timing and pattern of fusions occurring within vertebrae.

iii. RIBS AND STERNUM

INTRODUCTION

A characteristic of birds is the occurrence of ribs with vertebral and sternal segments both ossified. This may be a modification to assist in weight bearing during flight though it is also found in flightless birds (Bellairs and Jenkin 1960). Uncinate processes on the vertebral segments are also characteristic of most birds and may serve to strengthen the thoracic cage. Anasiewiczowna (1928) believed these to be rudiments of the superior ribs found in lower forms.

The last 2 cervical vertebrae carry relatively long costal elements termed ribs 1 and 2 by earlier authors. The true ribs are now considered to be the complete ribs i.e. having vertebral and sternal segments. The general view is that there are 5 pairs of these of which the sternal segment of number 5 does not directly reach the sternum but articulates with the rib anterior to it. 4 uncinate processes are described belonging

to the costal element of the last cervical vertebra and first 3 true ribs.

Ossification of the ribs was described by Knopfli (1919), Anasiewiczowna (1928), Schinz and Zangerl (1937), Hamilton (1952), Fujioka (1955) and Rumpler (1962). Romanoff (1960) gave a useful review of the earlier work. All agree that the ossification centres for all vertebral and sternal segments have appeared by hatching though quoted times vary somewhat. There is disagreement, however, on the time of ossification of the uncinate processes which is claimed by some to be prenatal and said to occur on the 17th day (Fujioka 1955, Rumpler 1962), 18th day (Schinz and Zangerl 1937) and 19th-20th day (Romanoff 1960). Others consider the ossification to be postnatal. Anasiewiczowna (1926) described the processes as still cartilaginous at hatching while Hamilton (1952) said they ossify postnatally but intramembranously.

The avian sternum was of early interest from the aspect of classification dividing birds into carinate, with a keel, or ratite, without. Ossification reports date from early authors like L'Herminier (1836a and b) who questioned the general view of the time that there were either 5 centres of ossification as in gallinaceous birds or 2 as in ducks. He considered there may be up to 9. L'Herminier (1836a) also provided one of the few reports on variation in ossification times in birds quoting times of sternal ossification of ducks in Guadeloupe as several weeks later than in France.

Several authors have described the development of the fowl sternum from paired mesenchymal and cartilaginous anlagen, and paired ossification centres for the body were described by various authors. Recent reports agree that the main ossification centre of the body is single

(Schinz and Zangerl 1937, Hamilton 1952, Fujioka 1955) confirming the view of Parker (1868). There is similar agreement that 2 pairs of centres are found for the sternal processes and that all centres appear prenatally.

Considerable variation in terminology, particularly regarding the processes, is encountered and is not resolved even in recent texts. Feduccia (1975) using a diagram based on Chamberlain (1943) marks one process but fails to identify it in the accompanying key, and gives a descriptive text for the processes which is quite unintelligible. King and McLelland (1975) use directionally descriptive terms for the processes and as these are at least clearly understandable they will be employed here. The terms applied are craniolateral and caudolateral processes the latter dividing into medial and lateral parts.

MATERIALS AND METHODS

6 Golden Comet pullets were killed within an hour of hatching. 3 were subjected to alizarin red S treatment and 3 were radiographed after impregnation with silver nitrate. From the same hatch 6 birds were killed at 7, 14 and 21 days postnatal and from each group 3 were similarly prepared with alizarin and 3 were radiographed after silver nitrate treatment.

RESULTS

A. Centres present at hatching

All 6 birds examined had 5 pairs of complete ribs, each with separate ossification centres for the vertebral and sternal segments (figs. 32-34) although the sternal segment of the 5th was very small in the bird shown

in fig. 32.

The uncinate processes of the costal element C16 and the vertebral segments of the first 3 true ribs had ossification centres variably present as shown in table 7.

LEFT			RIGHT		
RIB	+	-	RIB	+	-
C16	3	3	C16	3	3
T1	4	2	T1	4	2
T2	5	1	T2	4	2
T3	4	2	T3	3	3

Table 7 Numbers of birds showing ossification centres in uncinate processes at hatching.

In seemed that in the birds in this study the uncinate processes were ossifying just about the time of hatching and some variation was apparent. In 1 bird none had ossified and in 3 birds all had ossified. In the remaining 2 cases one bird showed centres in the processes of ribs T2 and T3 on the left only, while in the other centres had developed in the processes of ribs T1 and T2 on both sides, indicating that some variation in sequence can occur.

The centres for the uncinate processes are shown in figs. 32-34.

The sternal centres were all present showing 1 for the body and single centres in the paired craniolateral and caudolateral processes (figs. 32, 33).

B. Centres appearing postnatally

In the 18 birds examined all had 5 pairs of complete ribs except 1 in which the 5th pair lacked a sternal segment. There were centres in the 4 pairs of uncinate processes in all specimens, and all were ununited with the corresponding vertebral segment. No additional centres in ribs of sterna developed.

DISCUSSION

One variation in rib complement was encountered, namely a deficiency of the 5th pair of sternal segments. Herrick and Herrick (1956) found rib abnormalities in 10.4% of a large number of White Leghorn birds. The two basic abnormalities encountered were varying deficiencies of the 5th rib and the presence of an additional 6th rib. Complete absence of the sternal segment of the 5th is indicated as 1 variant of the first type. Marked reduction in the size of this segment is another quoted abnormality which would probably include the bird shown in fig. 32.

The variability of appearance of the uncinate process centres around the time of hatching is no doubt reflected in the divergence of opinion as to whether they are pre or postnatal in ossification. The variations quoted are rather wider than might have been expected although very little is known about variation in time of ossification in relation to breed sex or individuality. There is an indication that ossification of the uncinate process of the last cervical costal element may be slightly later than the others which has been claimed by Fujioka (1955).

iv. PECTORAL GIRDLE AND LIMB

INTRODUCTION/

INTRODUCTION

As described previously there is a clear general agreement that single centres of ossification are the rule in avian long bones, this being a characteristic of the class. There are also several reports detailing the prenatal times of appearance of the centres in the pectoral girdle and the wing skeleton in the fowl (Schinz and Zangerl 1937, Fujioka 1955, Romanoff 1960, Rumpler 1962). Single centres in the scapula, coracoid, humerus, radius and ulna are, therefore, known to be present by the time of hatching and the clavicles are known to be ossified as a single structure by that time.

The very reduced skeleton of the avian manus has given rise to a long standing controversy in comparative anatomy. The 3 rudimentary digits present in most species were generally believed to represent digits I-III of the primitive pentadactyl limb by the majority of authors until relatively recent times. Various contrary opinions were expressed but avian anatomical texts held fairly consistently to this doctrine. Montagna (1945) extensively reviewed the controversy and proceeded to reinvestigate the development of the carpus and manus in the domestic fowl. He described for the first time a mesenchymal condensation on the dorsoradial side of the wrist between centrale 1 and distal carpal II identifiable from the 6th - 9th day of incubation. This he considered to be distal carpal 1 representing the missing 1st digit. This primordium never chondrified but subsequently disappeared. Metacarpal I never appeared at all. The digits of the bird he therefore concluded to be numbers II - IV. A further investigation of the problem (Holmgren 1955) disagreed with some details of Montagna's findings but concurred with the general principle that the remaining digits are II - IV. This

is somewhat surprising as in an earlier paper (Holmgren 1933) the opposite view had been expressed. Like Holmgren the majority of authors subsequent to Montagna have adopted his interpretation the main exception being George and Berger (1966) who consider that the evidence is still inadequate. Montagna's view will be adopted in this study.

In the adult fowl 2 free carpal bones persist, traditionally termed radial and ulnar carpals. It is generally agreed that a distal row of 2 carpal bones eventually fuses to the proximal ends of the metacarpals forming the carpometacarpus, although some authors have erroneously described these as secondary centres of ossification in the metacarpus. The identity of the carpal elements was also reinvestigated by Montagna (1945), and Holmgren (1955) and the question reviewed by Romanoff (1960), largely adopting Montagna's findings. These were that the anlagen of the 4 definitive centres form about the 10th day of incubation by fusion of a number of prochondral primordia. These initially are grouped in 3 rows, proximal, central and distal. The proximal row consists of radiale, intermedium, ulnare and pisiform elements, the central row of centrale I - IV and the distal row of distale 1 - V making 13 elements in all. The "radial carpal" anlage is formed principally from centrale I plus the vestigial radiale and intermedium. The "ulnar carpal" is formed mainly from pisiform and vestigial ulnare. The "radial" and "ulnar" carpals are joined by a meniscus derived from centrale III. Of the 2 distal elements the one which fuses with the proximal end of metacarpal III is formed from centrale II and distal carpals II and III while the other, which similarly fuses to metacarpal IV is formed from centrale IV and distal carpals IV and V. The transient distal carpal I has been dealt with already.

The terminology of these definitive carpal elements presents a difficulty due to their complex origin. While recognising Montagna's findings it was decided to retain the time-honoured terms of radial and ulnar carpals for the proximal row and to term the distal row distal carpals III and IV on the basis that they fuse to the corresponding metacarpals.

The carpal centres of ossification appear postnatally (Schinz and Zangerl 1937, Fujioka 1955). Schinz and Zangerl quote times of appearance for 4 centres, a single centre for each definitive carpal element. Expressed in days postnatal these were:- radial 25-35, ulnar 0-12, distal carpal III 25-35, and distal IV 12-25.

The remaining metacarpals are II - IV. As described above metacarpal I is believed by Montagna (1945) never to appear at all. Metacarpal V he described as most rudimentary, fusing to the base of metacarpal IV and not found at all in the adult wing. Metacarpals III and IV were shown to ossify prenatally by Schinz and Zangerl (1937) and Fujioka (1955). Metacarpal II was said to be unossified at hatching (Fujioka 1955) and to ossify postnatally by 12 days (Schinz and Zangerl 1937) and by 2 weeks postnatal (Romanoff 1960). Metacarpal V was described by Schinz and Zangerl (1937) subsequently quoted by Romanoff (1960) as ossifying at 1 - 2 months postnatal but it is not clear whether this refers to a separate ossification centre.

In examining the literature concerning the phalanges of the domestic fowl and indeed birds in general yet another controversy was revealed regarding the numbers of phalanges present in the digits. The views of various authors are summarised in table 8. The digits are numbered according to Montagna (1945) regardless of the views of the original authors.

Author	Species Described	Dig II	Dig III	Dig IV	Comments
Newton and Gadow (1896)	Aves	2	2	1	Digit IV can be 2 in embryo
Kaupp (1918)	Gallus gallus	2	2	1	
Schinz and Zangerl (1937)	Gallus gallus	2	2	1	
Chamberlain (1943)	Gallus gallus	2	3	2	
Montagna (1945)	Gallus gallus	2	2	1	
Portmann (1950)	Aves	1	2	1	
Hamilton (1952)	Gallus gallus	2	3	2	In embryo distals of digits III, IV fuse to more proximal on 8th day
Sisson and Grossman (1953)	Gallus gallus	2	2	1	
Fujioka (1955)	Gallus gallus	2	2	1	
Bradley (1960)	Gallus gallus	2	2	1	
Bellairs and Jenkin (1960)	Aves	1	2	1	Can be 2:3:2 in some species and in embryo
Rumpler (1962)	Gallus gallus	2	2	1	
McLeod et al (1964)	Gallus gallus	2	3	2	
Lucas and Stettenheim (1965)	Gallus gallus	2	2	1	
George and Berger (1966)	Aves	1(2)	2(3)	1 (always)	
Evans (1969)	Melopsittacus undulatus	1	2	1	
King and McLelland (1975)	Gallus gallus	2	2	1	Other species have 2:3:2
Feduccia (1975)	Domestic species	1 (in most birds)	2	1	Additional distal phalanges rudimentary in embryo, subsequently fuse to more proximal

Table 8 Views of various authors concerning the numbers of phalanges in the digits of the manus.

Although it is clear from table 8 that the more popular view is that digits II - IV have 2:2:1 phalanges respectively, the formula of 2:3:2 crops up surprisingly frequently. The explanation is probably twofold. Several authors suggest that this may occur in species other than domestic fowl (Bellairs and Jenkin 1960, King and McLelland 1975). Secondly there are reports of additional distal phalanges occurring in embryo and fusing early to the more proximal phalanges (Newton and Gadow 1896, Hamilton 1952, Bellairs and Jenkin 1960).

However, neither of these explanations can account for the fact that Chamberlain (1943) shows a drawing of the skeleton of the manus in a fowl clearly indicating a formula of 2:3:2. The fusion of the carpometacarpus clearly shows this to represent an adult. The morphology of these additional distal phalanges is described in the text yet no comment is made as to whether they are an unusual occurrence. McLeod et al (1964) is so inaccurate in many respects that no great credibility can be ascribed to this work. Feduccia (1975) repeats the view that distal rudimentary phalanges may be present in embryo, subsequently fusing to the more proximal phalanges except in digit II where they remain free in the domestic fowl. Yet he very misleadingly reproduces the same illustration from Chamberlain (1943) without further comment. Furthermore, some of Chamberlain's legends are altered, again very misleadingly by naming proximal phalanges as digits. No mention is made of the inclusion of carpal elements in the carpometacarpus which is described as representing 3 fused elements, metacarpals II, III and IV.

The view that digit II may carry only 1 phalanx is found in several accounts but these appear to be mainly referring to other avian species, except Bellairs and Jenkin (1960) who show a diagram with just 1 phalanx

in the domestic fowl.

Ossification has been described as occurring prenatally in all phalanges and the timing given (Schinz and Zangerl 1937, Fujioka 1955, Rumpler 1962).

In this part of the study it was decided firstly to perform a survey on adult birds to investigate the normal phalangeal formula and then to confirm the centres present at hatching and those appearing postnatally.

MATERIALS AND METHODS

The adult survey was performed on 52 Golden Comet birds, 49 hens and 3 cockerels, aged 2½ years. After killing by intravenous injection of pentobarbitone sodium the left wing of each bird was radiographed laterally.

6 Golden Comet pullets were killed within 1 hour of hatching and 3 were prepared as alizarin red S specimens and the remaining 3 were radiographed after prior impregnation with silver nitrate.

Groups of 6 further birds from the same hatch were then killed at 7, 14 and 21 days postnatal and similarly treated. Thereafter 3 birds were sampled at 7 day intervals up to 112 days postnatal and then at 14 day intervals until 182 days postnatal. In these birds the left wing was radiographed laterally. It was found that after 21 days skeletal elements had adequate radiographic density to be identified without prior impregnation with silver nitrate.

RESULTS

A. Adult birds

The radiographs of the adult birds showed that a second phalanx in

digit II was present in 50 cases out of 52 (96.2%). Only 2 phalanges were found in digit III and one in digit IV in every case. The usual formula was, therefore, 2:2:1 but in 3.8% cases was 1:2:1.

B. Centres present at hatching

Single centres were present for the paired scapula, coracoid, humerus, radius and ulna and a single centre was found for the clavicle (figs. 35-37).

No carpal centres were present (figs. 38-39).

All birds showed single centres for metacarpals III and IV but in none had metacarpal II commenced ossification (figs. 38-39).

The 6 birds showed single centres in both phalanges of digits II and III (figs. 38-39).

1 of the 3 alizarin specimens (fig. 39) and 2 of the 3 radiographic specimens showed an ossification centre in the single phalanx of digit IV. The remainder did not (fig. 38).

C. Centres appearing postnatally

Single centres were found to appear postnatally in the 4 carpal elements and in metacarpal II (figs. 40-44). The occurrence of these centres is shown in tables 9 and 10.

Days Postnatal	0		7		14		21	
	L	R	L	R	L	R	L	R
Radial	-	-	-	-	+	-	+	+
	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-
Ulnar	-	-	+	+	+	+	+	+
	-	-	+	+	+	+	+	+
	-	-	+	+	+	+	+	+
Distal carpal III	-	-	-	-	-	-	+	+
	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-
Distal carpal IV	-	-	-	-	+	+	+	+
	-	-	-	-	+	+	+	+
	-	-	-	-	+	+	+	+
Metacarpal II	-	-	+	+	+	+	+	+
	-	-	+	+	+	+	+	+
	-	-	+	+	+	+	+	+

Table 9. Occurrence of postnatally developing centres of ossification - alizarin specimens.

Method	Silver nitrate radiography								Radiography			
Days Postnatal	0		7		14		21		28	35	42	49
Side	L	R	L	R	L	R	L	R	L	L	L	L
Radial	-	-	-	-	-	-	+	+	+	+	+	+
	-	-	-	-	-	-	+	+	+	+	+	+
	-	-	-	-	-	-	-	+	+	+	+	+
Ulnar	-	-	+	+	+	+	+	+	+	+	+	+
	-	-	-	-	+	+	+	+	+	+	+	+
	-	-	-	-	+	+	+	+	+	+	+	+
Distal carpal III	-	-	-	-	-	-	+	+	+	+	+	+
	-	-	-	-	-	-	+	+	+	+	+	+
	-	-	-	-	-	-	-	-	+	+	+	+
Distal carpal IV	-	-	-	-	+	+	+	+	+	+	+	+
	-	-	-	-	+	+	+	+	+	+	+	+
	-	-	-	-	+	+	+	+	+	+	+	+
Metacarpal II	-	-	+	+	+	+	+	+	+	+	+	+
	-	-	+	+	+	+	+	+	+	+	+	+
	-	-	+	+	+	+	+	+	+	+	+	+

Table 10 Occurrence of postnatally developing centres of ossification - radiographic specimens.

The centre for metacarpal II was seen in all birds examined at 7 days and over. The ulnar carpal was ossified in some 7 day old birds and in all thereafter, and distal carpal IV in all aged 14 days and over. The radial carpal centre was first seen at 14 days and in all aged 28 days or more while distal carpal III was first apparent at 21 days and in all thereafter.

In all birds aged 7 days or over the single phalanx of digit IV was ossified.

The ranges of time of appearance of these centres were calculated as before and are shown in fig. 20a and their mean times of appearance are shown in fig. 20b.

DISCUSSION

The adult survey may explain the controversy over the number of phalanges in digit II which would appear to be truly variable. Whether the occasional reduction is a result of fusion of pre-existing elements and at what stage this might occur is impossible to say. There was no indication of additional distal phalanges for digits III and IV.

The centres present at hatching agreed with the findings of Schinz and Zangerl (1937) and Fujioka (1955) except for the phalanx of digit IV which was found ossified in 3 of the 6 birds examined at hatching though it was present in all thereafter. Although these authors considered it prenatal in appearance the times quoted are very close to hatching, 20 days (Fujioka 1955) and 21 days (Schinz and Zangerl 1937).

A single centre found to appear in each of the 4 carpal elements was of no value in elucidating the identities of these, the fusion of the multiple primordia having occurred at a very much earlier stage.

Compared to the range of time of appearance of the carpal centres quoted by Schinz and Zangerl (1937) the ranges in this study were rather earlier for the radial, very similar for the ulnar and slightly earlier for the distal carpals. Metacarpal II was found ossified in all cases by the 7th day, slightly earlier than quoted by Schinz and Zangerl (1937) and Romanoff (1960).

v. PELVIC GIRDLE AND LIMB

INTRODUCTION

The pelvic girdle has been shown to ossify from single centres for the paired ilium, ischium and pubis and the pelvic limb to have single centres for the diaphyses of the long bones, femur, tibia, fibula, metatarsals and all phalanges by hatching (Schinz and Zangerl 1937, Hamilton 1952, Fujioka 1955, Romanoff 1960, Rumpler 1962). Whether the fusion of the centres for metatarsals II, III and IV has been accomplished by this time is not totally clear either from the descriptions or the illustrations provided.

The characteristic lack of secondary ossification centres in avian long bones has been alluded to already. It is in the pelvic limb that most claimed exceptions to this are found.

Much debate has ensued over reports by various authors regarding the presence or absence of "epiphyses" in various avian long bones. However, a considerable part of this alleged conflict of opinion centres around the use of the term "epiphysis" rather than real difference in opinion on the occurrence of secondary centres. The term "epiphysis" is applied by some to the growing cartilaginous end of a long bone which may or may

not contain a centre of ossification, while others define it as the secondary centre of ossification occurring in the extremity of a long bone. The latter definition is in accordance with B.N.A. and being so familiar to many workers has led them to misunderstand the term as used by other authors. The B.N.A. definition is somewhat misleading as it refers to a structure which is not in fact a typical long bone feature in tetrapods, as these secondary centres are of regular occurrence only in the mammal and of occasional occurrence only in some reptiles and birds as will be discussed later. There can, therefore, be little justification for this use of the term in comparative anatomical studies.

When Parsons (1904) referred to a "true epiphysis" at the upper end of the tibia in gallinaceous birds he was referring to a secondary centre of ossification and his classification of epiphyses into pressure, traction and atavistic types refers to types of these ossification centres.

Fell (1925) has been wrongly accused by Church and Johnson (1964) of being equivocal in her discussion on the occurrence of avian epiphyses, by saying that long bones have epiphyses which are unossified. She was merely redefining the term to exclude the necessary presence of a secondary ossification centre.

Latimer (1927) talked of <u>epiphyseal lines</u>, presumably meaning growth cartilage plates but only described a separate ossification centre at the distal end of the tibia. He was subsequently misquoted by Haines (1942) as having claimed to find <u>epiphyseal centres</u> in all the long bones. In fact Haines himself had made the distinction between epiphyses as growing regions and as secondary ossification centres in an earlier paper (Haines 1938) and made the point that epiphyseal structures as a whole are phylogenetically much older than secondary centres.

In the investigation of the occurrence of secondary centres of ossification in this study they will be referred to as such rather than as epiphyses.

When the literature is searched purely for evidence of secondary centres in the long bones of birds the position is considerably clearer, though there is still some disagreement and the patently inaccurate statements of Chamberlain (1943) and McLeod et al (1964) have been mentioned already.

It is well known that there are 2 rows of ossification centres at the hock joint, the proximal becoming fused to the tibia and the distal to the great metatarsal. The earliest investigators claimed these to represent the tarsal elements (Owen 1866, Morse 1872) and the view is still maintained, though the actual identities of these tarsal centres are far from clear (Holmgren 1955). The final fusion of these elements to the adjacent long bones produces the bones usually termed tibiotarsus and tarsometatarsus indicating the compound origin of these elements. When these terms are employed there can be no justification for regarding the tarsal elements as secondary ossification centres of the tibia and great metatarsal as has been done by Church and Johnson (1964) and Franceschini (1967). Indeed the latter author, although his is one of the most recent investigations on the subject, makes no reference to the tarsal identity of the "distal epiphyseal centre" of the tibia and even says that the tibia is the only bone in the fowl skeleton to possess secondary centres. It is hard to see how he manages to exclude the metatarsus from this.

The numbers of these tarsal elements has been disputed particularly in recent reports. There are usually described 2 in the proximal row

(Bruce et al. 1946, Fujioka 1955, Nielson 1963. Church and Johnson 1964. Varicak 1968, Navagiri 1975) though Schinz and Zangerl (1937) have qualified this by describing the 2 centres as being formed by the fusion of 4 and 2 elements respectively shortly after their appearance. A single centre has been claimed (Rumpler 1962) and 3 centres claimed by Franceschini (1967). This latter report describes an additional 3rd centre in the cartilaginous cone projecting upward between the regularly described medial and lateral centres into the distal end of the tibia. The method used was serial section and histological examination unlike most investigations which have been by alizarin or x-ray. The illustrations are far from convincing, indeed one legend refers to a "hint of a 3rd centre in the cartilaginous cone". There are also some surprising gaps in the cited literature even the widely quoted report of Schinz and Zangerl (1937) which deals with these centres in some detail.

The time of ossification of these proximal tarsal centres has also been the subject of some dispute, being described as prenatal (Fujioka 1955, Navagiri 1975) around hatching (Schinz and Zangerl 1937, Nielson 1963) and postnatal, soon after hatching (Church and Johnson 1964) and at about 1 week (Franceschini 1967) this being the quoted time for their 3rd centre also.

The distal tarsal has been described as a single centre by most authors but as 2 centres rapidly fusing to 1 by Schinz and Zangerl (1937). Its time of ossification has been given as prenatal (Fujioka 1955) around hatching (Schinz and Zangerl 1937) and soon after hatching (Church and Johnson 1964).

The centre at the proximal end of the tibia has been described by Parsons (1904), Haines (1942), Varicak (1950), Frank (1962), Church and

Johnson (1964), Franceschini (1967) and Varicak (1968). Its exclusion from the various accounts of ossification is probably explained by its relatively later time of appearance as these studies have concentrated on the embryonic period.

In the fowl this centre has usually been described as single except by Rumpler (1962) who is claimed by Franceschini (1967) to have described it as paired. In fact Rumpler's meaning is doubtful but seems to refer to 2 superior centres of the metatarsus.

The quoted times of appearance are 5 weeks postnatal (Church and Johnson 1964, Franceschini 1967), and from 6 weeks upwards (Frank 1962).

Typically in the mammal there are 2 centres of ossification at the proximal end of the tibia of which one belongs to Parson's group of "pressure epiphyses" and the other, which forms the tibial tuberosity and is closely associated with the attachment of the patellar ligament, is a "traction epiphysis". The single centre in the avian tibia has usually been referred to simply as the proximal tibial centre and little attention paid to its identity beyond this.

The aim of this part of the study was to confirm centres present at hatching and appearing subsequently and to enquire into the identity of the proximal tibial centre.

Sesamoids are not included here, being dealt with under a separate heading.

MATERIALS AND METHODS

6 Golden Comet pullets were killed within 1 hour of hatching. 3 were prepared as alizarin red S specimens and 3 were radiographed after prior treatment with silver nitrate. Thereafter groups of 6 birds from

the same hatch were killed at 7, 14 and 21 days postnatal and similarly treated. After this groups of 3 were killed at 7 day intervals till 112 days and then at 14 day intervals till 182 days and the left pelvic limbs radiographed laterally and craniocaudally without previous silver nitrate impregnation.

The left pelvic limb of a 70 day old pullet and a 5 month pup of similar size were removed immediately after death and the stifle joints radiographed laterally. The long bones were then sectioned longitudinally through the stifle joint after prior freezing. The sectioned surfaces were then examined and photographed.

RESULTS

A. Centres present at hatching

These were single centres for the paired ilium, ischium, pubis, diaphyseal centres of paired femur, tibia, fibula, metatarsal I, metatarsals II, III and IV which were fused together and single centres for phalanges of digits I - IV which numbered 2, 3, 4 and 5 respectively. 3 tarsal centres were present, 2 proximal and 1 distal (figs. 35-37, 45-49).

B. Centres appearing postnatally

The proximal tibial centre was first found ossified in 1 bird at 56 days and in all birds examined thereafter. The range of time of appearance is shown in fig. 20a and the mean time of appearance in fig. 20b.

No further centres were found to appear.

C. Identity of proximal tibial centre

The sectioned surfaces of the 70 day old fowl and pup tibias were compared (figs. 50a, 50b). The single centre at the proximal end of the fowl tibia was contrasted with the 2 separate centres in the pup. Below the centre for the tibial tuberosity of the pup was an outgrowth from the diaphysis for the tibial crest which can be termed an apophysis. In the fowl the anterior limit of the proximal centre was situated anteriorly almost as far as the "traction epiphysis" of the pup. However it extended further posteriorly. In the fowl the centre projected markedly upwards to a point into which the patellar ligament attached. In the pup the "traction epiphysis" sat rather more inferiorly being accommodated in a notch on the anterior aspect of the diaphysis. The patellar ligament attached to the "traction epiphysis" (figs. 50a, 50b). On the radiographs (figs 50c-d) the anterior location of the fowl's proximal tibial centre was even more obvious. In the pup the faint shadow of the patellar ligament is seen indicating very strikingly its direction of action from patella to "traction epiphysis" to tibial diaphysis. The patella had not yet ossified in the fowl.

DISCUSSION

The study largely confirmed the findings of Schinz and Zangerl (1937) and Fujioka (1955) concerning the centres present at hatching, 3 single tarsal centres were found at that time.

The only centre found to appear postnatally was the proximal tibial centre and was found rather later than in previous reports. This proximal tibial centre was the only secondary centre found in the fowl skeleton. The tarsal centres probably appeared just prior to hatching and the carpal

centres appear in the first month or so after hatching (vid. sup.). Thereafter there is a gap until the proximal tibial centre ossifies (fig. 20b) this having the later time of appearance characteristic of secondary centres.

Haines (1938) claimed that secondary centres are found only in mammals, lacertilia (lizards) and in the avian tibia, and that the cartilage canals found in mammals are usually associated with the subsequent development of a secondary centre. This is similar in lizards. Cartilage canals are present in the epiphyses of birds and this is taken by Haines to indicate that at one time they possessed more secondary centres which may have disappeared with the invasion of air sacs causing pneumatisation. This speculation is unlikely to be well founded, as widespread pneumatisation of the long bones is unusual.

The patellar ligament was attached to the proximal tibial centre as it was to the "traction epiphysis" of the canine tibia. The lateral radiograph of the tibia of the 70 day old fowl (fig. 50c) indicated that the relative position of the centre shortly after its initial appearance resembled the "traction epiphysis" rather more than the pressure epiphysis of the mammal. For these reasons it is believed that this represents a "traction epiphysis" associated with the insertion of the patellar ligament. The centre did appear to extend rather more posteriorly than the corresponding centre in the pup but this may be accounted for by the lack of another centre to inhibit its progressive ossification through the cartilage.

Parsons (1904) suggested that traction epiphyses may represent pre-existing sesamoids. Haines (1940, 1942) disagreed and termed them

intratendinous centres developed as direct ossifications of tendons where they insert into cartilaginous epiphyses and believed them separate in their origin from sesamoids. Barnett and Lewis (1958) supported Parson's theory since in some birds the patella may be absent having fused into a very large tibial crest, while in cormorants according to Portmann (1950) the tibial crest is detached, behaving, therefore, much like a sesamoid.

The question may well be posed, why should the avian skeleton contain this solitary "traction epiphysis"?

In birds the patella is often a well developed, thick structure and there is frequently present at the hock joint a tarsal sesamoid. These facts, and the frequent occurrence of calcification or ossification of the pelvic limb tendons would suggest that these tendons may be subject to considerable stress forces, particularly when landing from flight, in the bipedal posture of birds. The proximal tibial centre may represent a fusion of a pre-existing sesamoid as a further strengthening device for the insertion of the quadriceps femoris muscle. Such a sesamoid would have been particularly closely related to the tibia, especially due to the avian bipedal posture where the long axis of the femur is much nearer the horizontal than is the case in man. It is not difficult therefore to imagine it readily becoming fused on to the tibia. The markedly upward projection of the centre may be a further refinement resulting from this particular bipedal posture in that it may permit a more direct course for the tendon, avoiding an acute terminal reflection.

vi. LIMB TENDONS AND SESAMOIDS/

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INTRODUCTION

The tendency for the limb tendons to undergo ossification is a characteristic of the class aves (Evans 1969). More specifically it has been claimed that many of the long tendons of the shank and foot and of the antebrachium and epaxial musculature develop extensive centres of mineralisation with subsequent ossification (George and Berger 1966, Vanden Berge 1975). This phenomenon is said to be most common in galliform birds (Hudson et al. 1959) but little is known of its occurrence in individual muscles or of its function, and very little regarding the timing of development.

Regarding sesamoids information is also very sparse. The patella is known to occur in many species and may become fused to the tibial crest or may be double as discussed previously. The time of ossification has been quoted as 11 weeks after hatching (Niven 1933).

A tarsal sesamoid is described by many authors and well shown by Chamberlain (1943). Curiously it is not mentioned in several recent accounts (Sisson and Grossman 1953, King and McLelland 1975, Feduccia 1975). There is sometimes confusion between this and the hypotarsus, which term is properly applied to the process at the superoposterior part of the tarsometatarsus which is grooved or even converted into a canal for the passage of flexor tendons. Fujioka (1955) shows a diagram with what resembles the tarsal sesamoid marked as the hypotarsus which he says is ossified postnatally. Chamberlain (1943) marks the sesamoid as the hypotarsal sesamoid in a figure but terms it the medial tarsal sesamoid in the text which by an apparent error is also cross referenced to

the hypotarsal ridge in another figure.

Other references to sesamoids are very occasional. Bellairs and Jenkin (1960) mention an os humeroscapulare as sometimes present between head of humerus and scapula with some deltoid fibres attaching to it and say that sesamoids may occur at the elbow. George and Berger (1966) describe a sesamoid in the tendon of scapulotriceps which they term the patella ulnaris. None of these reports refers specifically to the domestic fowl.

Hudson et al. (1959) describe sesamoids in the pelvic limb of many species including Gallus gallus in which they describe 1 in the shank and 8 in the tarsus. It is not clear, however, whether these are actually ossified or just cartilaginous nodules.

This part of the study aimed to survey the occurrence of centres for tendons and sesamoids in the adult bird and to investigate the timing of their development.

MATERIALS AND METHODS

For investigation of the adult situation 52 adult Golden Comet birds aged $2\frac{1}{2}$ years, 49 hens and 3 cockerels were used. They were killed and lateral view radiographs of left wing and pelvic limb taken.

The investigation of timing of the development of centres of mineralisation was performed on 2 groups of birds which were killed in groups as follows:-

1. White Leghorns

   1 male and 1 female at hatching then similarly at 7 day intervals till 119 days postnatal and the last male bird at 126 days.

ii. Golden Comets

3 females at hatching, then at 7 day intervals until 112 days then at 14 day intervals until 182 days.
Lateral radiographs of the left limb of all birds were taken. Those up to and including 21 days of age were first impregnated with silver nitrate to increase their skeletal density.

RESULTS

A. Centres present in the adult

i. Tendons

In the wing these were recognised singly on the dorsal aspect of the metacarpus and on the palmar aspect of the antebrachium (fig. 51). In the pelvic limb they were seen singly on the plantar aspect of tibiotarsus, on the dorsum of the tarsometatarsus and occasionally on the dorsum of the tibiotarsus. On the plantar side of tarsometatarsus several were found probably up to 5 though the exact number was difficult to determine on account of superimposition. These are shown in fig. 52.
The frequency of occurrence of these tendons is shown in table II.

Tendons	Frequency	% Age	Notes
Dorsal Tarsometatarsal	52	100	3 cases showed division into 2 at distal 1/3 of tarsometatarsus
Plantar Tarsometatarsal	52	100	In 22 cases 4 and in 30 cases 5 were estimated to be present
Dorsal Tibiotarsal	2	3.8	Both were cockerels
Plantar Tibiotarsal	33	63.5	1 bird showed 2 tendons
Dorsal Metacarpal	39	75.0	
Palmar Antebrachial	8	15.4	

Table 11 Incidence of mineralised tendons in 52 adult birds.

ii. Sesamoids

Patellar and tarsal sesamoids were present in all cases. The tarsal sesamoid is shown in fig. 52. A small sesamoid was recognised on the dorsal aspect of the carpus close to the radial carpal bone in 33 cases (63.5%). This was termed the dorsal carpal sesamoid and it is shown in fig. 51. No other sesamoids were identified.

B. Centres present at hatching

None.

C. Centres appearing postnatally

i. Tendons

The occurrence of these is shown in table 12.

Tendons	Birds	Up to 84 days	91	98	105	112	119	126	140	154	168	182
Dorsal Tarsometatarsal	WLM	0	0	0	0	0	1	1	x	x	x	x
	WLF	0	0	0	1	1	0	x	x	x	x	x
	GC1	0	0	0	0	1	x	1	1	1	1	1
	GC2	0	0	0	0	0	x	1	1	1	1	1
	GC3	0	0	0	0	1	x	1	1	1	1	1
Plantar Tarsometatarsal	WLM	0	3	0	3	3	4	5	x	x	x	x
	WLF	0	3	0	4	4	4	x	x	x	x	x
	GC1	0	4	0	0	4	x	5	5	5	5	5
	GC2	0	2	2	2	4	x	5	5	5	5	5
	GC3	0	2	3	2	5	x	5	5	5	5	5
Dorsal Metacarpal	WLM	0	0	0	1	1	x	1	x	x	x	x
	WLF	0	0	0	1	1	x	x	x	x	x	x
	GC1	0	1	0	0	0	x	1	0	0	0	0
	GC2	0	1	0	0	0	x	1	0	0	0	0
	GC3	0	0	0	0	0	x	1	0	0	0	0

WLM White Leghorn males

WLF White Leghorn females

GC1-3 Golden Comet females

x not examined

Table 12 Numbers of mineralised tendons identified in growing birds of known ages.

The first were identified at 91 days postnatal these being the plantar tarsometatarsals in all birds and that on the dorsum of the metacarpus in 2 Golden Comets. Thereafter, the plantar tarsometatarsals were regularly present other than in 3 instances, the number being variable but increasing up to 126 days when 5 centres appeared to be present although again it was difficult to be certain of the exact number, if this exceeded 3 due to superimposition.

A mineralised tendon on the dorsum of the tarsometatarsus was first seen in a White Leghorn female bird at 105 days and at 112 days in 2 Golden Comets. Thereafter it was of regular occurrence in the Golden Comets. The dorsal metacarpal tendon after its initial identification showed a variable occurrence in line with its occurrence in the adult survey. The remaining tendons found to be mineralised in the adult survey did not develop centres of mineralisation during the period of the investigation.

ii. Sesamoids

The centres for the patella and the tarsal sesamoid were first seen at 63 days postnatal in a White Leghorn female bird. They were identified at 70 days in both White Leghorns and in all thereafter. In the Golden Comets these centres were found in 1 bird at 77 days in none of the 84 day olds and in all birds aged 91 days and over.

The dorsal carpal sesamoid was not identified throughout this investigation.

The ranges of the time of appearance of the centres for the

sesamoids and tendons are shown in fig. 20a and their mean times of appearance in fig. 20b. The tendon on the dorsum of the metacarpus is excluded, being too variable in occurrence to enable its range of time of appearance to be accurately determined from the data available.

DISCUSSION

This part of the study was performed to complete the picture of ossification centres rather than to provide exhaustive investigation of the occurrence of sesamoids and mineralised tendons. It does, however, provide useful information on a number of points.

The tarsal sesamoid was of regular occurrence and there would seem to be no good reason for its exclusion in texts.

In addition to these the dorsal carpal sesamoid was the only one identified. This does not seem to have been described previously. A radiographic study provides no information, of course, on incidence of cartilaginous sesamoids which might yet ossify later, the birds in the adult survey being only $2\frac{1}{2}$ years old.

The times of appearance of the patella and tarsal sesamoid were remarkably uniform even between the 2 groups of birds, although this is well into the postnatal period. This is very different from the findings of a study on postnatally ossifying sesamoids in calves by Lindsay et al. (1969, 1973). The times of appearance of the tendinous centres also showed surprisingly little variation.

Vanden Berge (1975) described mineralisation and subsequent ossification of limb tendons. In this study care has been taken to refer to centres of mineralisation rather than ossification. Examination of

aged macerated tarsometatarsal specimens often indicates ossified tendons fused to the bone. No evidence of fusion was seen in the birds in this study. It remains to be decided when actual ossification of the tendons occurs. Other points arising from this study which need further attention are the times of mineralisation of the dorsal carpal sesamoid and tendons which had not developed their centres by the end of this study. Further work is also needed to discover the individual identity of the various mineralised tendons.

vii. LARYNX AND TRACHEA

INTRODUCTION

The cartilages of the larynx and trachea are frequently described as eventually undergoing ossification (Kaupp 1918, Bradley 1960, King and McLelland 1975 and others).

Details of the distribution of ossification in the laryngeal cartilages were given by White (1975), who described the cricoid as extensively ossified, except for the rostral shovel shaped portion of the median body and the most caudal part of its wings and their junction with the body. The procricoid she described as almost completely ossified and the arytenoid in its body only. The pattern of development of ossification of these cartilages was studied by the alizarin red S method (White 1970).

The tracheal rings tend to ossify in large species (King and McLelland 1975) and this is said to occur in the ventral parts of the rings (Bradley 1960). Garside (1968) described ossification of the tracheal rings in 200 birds examined histologically and claimed it occurred only from 15 weeks upwards. It commenced in the lower rings and spread craniad.

The syrinx as defined by Myers (1917) included the last 4 tracheal rings these being the cranial syringeal cartilages or tympanum, the 4 incomplete intermediate cartilages, the caudal syringeal cartilages which are the first 3 bronchial half rings and the pessulus in its skeletal structure. The attachments of the 1st bronchial cartilage were said to become ossified in old age but the other bronchial cartilages to remain unossified. King (1975) described 4 cranial syringeal cartilages in the male and 3 in the female of which the first 2 were ossified as is the pessulus and the ventral end of the first caudal syringeal cartilage, but the intermediates were not.

It was decided to investigate the extent of ossification in the adult larynx, trachea and syrinx and follow its pattern of development.

MATERIALS AND METHODS

The larynx, trachea and primary bronchi as far as the hilus of the lung were removed from Golden Comet pullets in pairs at hatching and at 7 day intervals to 112 days, then at 14 day intervals till 182 days postnatal. 2 adult birds aged $2\frac{1}{2}$ years were also sampled. The specimens were treated with alizarin red S and inspected under the operating microscope. The distribution of the alizarin reaction was recorded in the laryngeal cartilages, in the tracheal cartilaginous rings which were counted and recorded individually and in the syringeal skeleton.

After it was established that the alizarin reaction was strongly developed in the older birds in the series, portions of cricoid cartilage, and a caudal tracheal ring were removed from birds aged 154, 168 and 182 days and from an adult. These were sectioned and stained with haematoxylin and eosin for histological examination.

RESULTS

1. Larynx

The occurrence of mineralisation in the laryngeal cartilages was recorded in the same manner as by White (1970) and is shown in fig. 53.

Mineralisation was first seen at 105 days when it was found bilaterally in the arytenoids of 1 specimen. It was next seen at 126 days in 1 bird as a paired centre in the cricoid body (fig. 54) and in the other was widespread in the cricoid body. Both birds also showed it in the cricoid wings, one completely and the other as paired centres (fig. 55). The procricoid was completely involved in both as was the body of the arytenoids (fig. 55).

Thereafter mineralisation was observed in all specimens in all cartilages, and was complete in all cricoid wings, procricoids and arytenoids, except their processes. In the cricoid body the extent was variable and never extended into the rostral process or the most caudal part.

The adult pattern was very similar in both birds. This extent was first reached in a bird aged 140 days. The distribution in all specimens tended to be fairly bilaterally symmetrical. Fig. 56 shows the fully developed pattern in a bird of 154 days.

Histologically up to 182 days the cricoid consisted of cartilage in which the cells showed marked degenerative changes and were very clearly grouped into bands. The ground substance was markedly basophilic, very in some regions. No vascularity was detected, however, and no actual bone tissue was observed (fig. 57). In the adult traces of bone tissue were found but large amounts of mineralised

cartilage still remained.

The range of time of appearance of the centres of mineralisation in the laryngeal cartilages is shown in fig. 20a and their mean times of appearance in fig. 20b.

ii. Trachea

In 18 birds examined during the period when mineralisation occurred the total number of rings to the terminal bifurcation varied from 107 - 121. The pattern of mineralisation is shown diagrammatically in fig. 58. The following points are noteworthy.

Mineralisation was first observed in 1 bird at 98 days then in 1 at 105 days, 1 at 112 days and in all examined thereafter.

It commenced at the caudal end of the trachea and spread cranially, the caudal rings rapidly becoming fully involved with the exception of the last which was not mineralised except partially in the adults and the penultimate which frequently was only partially involved (figs. 59a, b). The first ring was not involved until 126 days. There was no case in which every ring was involved even in the adults.

Some single isolated rings were unmineralised and all unmineralised rings were adjacent to rings with no more than 25% mineralisation almost without exception other than the last rings, and in general the extent of mineralisation was similar throughout a group of adjacent rings.

The cranial half of the trachea nearly always contained rings not more than 50% mineralised and often less. The most cranial ring found to be 75% affected was no. 51 at 126 days and the most cranial ring fully mineralised was no. 83 in an adult.

Rings often contained 2 or 3 individual centres (fig. 60).

Up to 154 days the histological appearance was of cartilage showing marked basophilia of the matrix and marked transverse banding of the cells which showed various degrees of degeneration. Foci of marrow-like cells were seen but no actual bone deposition (fig. 61). At 182 days and in the adult cancellous bone tissue was observed with numerous foci of marrow (fig. 62).

iii. Syrinx

The cranial syringeal cartilages forming the tympanum have been dealt with under the heading of trachea.

The alizarin reaction occurred in the pessulus and the most ventral part of the first caudal syringeal cartilage and was always found simultaneously, but not in the intermediates or other bronchial cartilages (figs. 59a, b). It was found first in 1 bird of 98 days and in all examined subsequently. The whole of the pessulus was always found to be involved.

The range of time of appearance of the centres for the pessulus and 1st caudal syringeal cartilage are shown in fig. 20a and their mean times of appearance in fig. 20b.

DISCUSSION

The extent of mineralisation in the adult larynx was very similar to that shown by White (1970) as ossification. The developmental pattern was basically similar. Points of difference were that in this study the onset of mineralisation was first seen in the arytenoids at 105 days as against 84 days in the cricoid body. The procricoid was found rather earlier than by White (1970) along with the other centres.

The tracheal rings including those forming the tympanum numbered 107-121 in 18 birds. If 3 is deducted being the number of cranial syringeal rings present in the female (King 1975) a range of 104-118 is obtained having a slightly lower limit than the range quoted for 10 birds by McLelland (1965), who counted the tracheal rings in this way.

The onset of mineralisation at 98 days agreed very closely with Garside (1968) as did the initial site in the caudal rings and the cranial direction of spread of the process.

Although the alizarin reaction occurred from 98 days onwards and was very intense and diffuse in the caudal tracheal rings this was apparently due purely to the presence of mineralised cartilage and true bone was found only in the trachea of the oldest birds in the growing series and in larynx and trachea of adults. Garside (1968) described vascularisation and deposition of bone tissue as occurring "later" than the mineralisation of cartilage but the time interval is not specified.

It would seem therefore that there is a considerable interval between the initial onset of mineralisation as detected by the alizarin reaction and the development of ossification. The presence of marrow foci in advance of true ossification would appear to be similar to the situation in man noted by Summerfield King (1963).

The oldest birds in Garside's series were 2 years old, and in them he described the process as still progressing and says it is not known at what age ossification of all rings is completed. In this study the adult birds examined were $2\frac{1}{2}$ years old and all rings were by no means fully mineralised. Indeed the extent of mineralisation found in adults had been attained by birds of 140 days of age, and it may be that this is in fact the maximum extent achieved.

It was suggested by Myers (1917) that the cranial syringeal rings, being relatively thick and closely bound together, may form a functionally important rigid zone of the syrinx. Their early and complete mineralisation would tend to confirm their rigid nature except in the case of the last which remains unmineralised other than in the adult and resembles the intermediate cartilages in this respect. This may provide additional flexibility for the external tympaniform membranes.

CONCLUSIONS FROM PART 1 OF STUDY

Many of the results of this study confirm some of the earliest investigations on the subject some of which such as those of W.K. Parker are remarkable for their accuracy. Several of the many existing controversies have in fact been introduced in the relatively recent literature.

The centres of ossification present at hatching and those appearing postnatally have been identified, named and illustrated. Many agree with previous reports but the following points are of significance:-

i. At hatching there was no evidence of the multiple centres of ossification from which many of the skull bones develop. The separate identities of the parasphenoid and basisphenoid were not identifiable nor were the epiotic and opisthotic which were presumed to have fused in the prenatal period to adjacent elements.

ii. A paired orbitosphenoid was identified. No presphenoid was found.

iii. In the mandible the dentaries were found to be fused into 1 single unit at hatching.

iv. The entoglossal had initially a small paired centre of ossification. All hyoid elements except the ceratobranchial ossified postnatally.

v. The atlas had 3 and the axis 5 centres. The remaining vertebrae had 3 basic centres, 1 for centrum and 2 for arch though by hatching varying stages of fusion of these had already occurred. Additionally there were separate centres at hatching for costal elements (excluding vertebral ribs) in cervicals 3-16, lumbosacral 8 (variably) and in lumbosacral 9 where there was sometimes fusion to the transverse process. Transverse processes with separate centres were found in the region lumbosacral 6-9, no bird having more than 3 pairs.

vi. The phalangeal formula in the manus was normally 2:2:1 but in a few cases was 1:2:1.

vii. 4 carpal centres were found, all ossifying postnatally.

viii. Metacarpals II, III, and IV each had a single centre but metacarpal V did not and was not even recognisable as a prolongation of metacarpal IV.

ix. 3 tarsal centres were recognisable at hatching, 2 proximal and 1 distal.

x. The proximal tibial centre was the only true secondary centre in long bones and represented the mammalian tibial tuberosity centre being of the "traction epiphysis" type.

xi. The patella and tarsal sesamoid were constant centres in adults, and a dorsal carpal sesamoid was variable. No other ossified sesamoids were found.

xii. Mineralised tendons were found in adults in the regions, dorsal tarsometatarsus, plantar tarsometatarsus (4-5 or more), dorsal tibiotarsus (cockerels only), plantar tibiotarsus, dorsal metacarpus and palmar antebrachium (low incidence).

xiii. Mineralisation was found to develop in all laryngeal cartilages and to become widespread in the tracheal cartilages. The involvement was always greatest in the caudal region except the last ring which was only involved in the adults. All cartilages were never completely involved even in adults. The pessulus and base of the first bronchial cartilage were also involved. True ossification only commenced at the end of the growing series.

The timing of the appearance of the postnatally developing centres has been shown. The variation found was not great even with the centres appearing relatively late such as sesamoids. No difference was observed between the methods of alizarin staining and radiography and little when comparison was possible with the White Leghorn male and female birds.

The centres for the uncinate processes and the single phalanx of digit IV appeared around hatching. The carpal centres and that of metacarpal II were found to develop in the neonatal period. Thereafter there was a substantial interval until the next group of centres appeared which included the one true secondary centre, (the proximal tibial), the hyoid centres other than the ceratobranchial, orbitosphenoid, patella and tarsal sesamoid. The final group were the centres of mineralisation for some tendons, and the laryngeal, tracheal and syringeal cartilages. Other tendons and the dorsal carpal sesamoid which were mineralised in the adult did not develop centres during the growth period.

PART TWO

FUSION IN THE POSTNATAL SKELETON

GENERAL INTRODUCTION

"In the bird class we meet this remarkable phenomenon, namely that the swiftest creatures by far that inhabit the earth have had for the purposes of their most consummate mechanism the greatest loss of freedom of the individual parts of the skeletal framework".

In these words W.K. Parker (1887) drew attention to one of the most remarkable evolutionary modifications in the animal kingdom. Young (1950) has expanded this theme by remarking how superimposition of extensive fusion on the basic skeletal plan of the bipedal archosaurian reptile has resulted in the group of animals probably best suited to locomotion by 2 distinct means i.e. flight and walking, in addition to which many can swim.

During flight the body weight is suspended from the wings and is transmitted through a relatively rigid fused vertebral column, completely ossified ribs and a large fused sternum. In walking the situation is reversed in that the body weight is transmitted to the pelvic limbs as is usual with a bipedal posture but in addition birds have to cope with the substantial added forces generated during landing from flight. The extensive synsacral fusion and the widespread area of union between the synsacrum and the expansive pelvic girdle would appear to be valuable modifications in this respect.

Headley (1895) drew attention to the particular problems engendered by assuming a bipedal posture with the trunk still in an almost horizontal position. It would seem that the fusion of the vertebral column has evolved in preference to the development of additional epaxial musculature to cope with this situation. Any additional muscle mass would have had the effect of increasing body weight and indeed there is

in association with vertebral column fusion a reduction in epaxial muscle quantity. Thus the 2 main muscular groups in birds are those directly associated with its 2 modes of locomotion, the pectoral mass for flight and the pelvic limb musculature for terrestrial movement.

Other examples of fusions as modifications for flight are associated with the development of firm bases for feather attachment. Thus the total commitment of the pectoral limb to flight has been accompanied by extensive fusion of the bones of the manus for a stable foundation for the primary remiges and the terminal coccygeal vertebrae have fused to form the pygostyle which supports the retrices.

Another skeletal adaptation for flight is the general reduction in bone density coupled with pneumatisation of various regions, yet the muscles of locomotion are extremely powerful and, as suggested by George and Berger (1966), fusion may also be a device to compensate for the relative fragility of the bird skeleton.

Extensive fusion of the skull has been claimed as a necessary prerequisite for the development of widespread pneumatisation. This topic will be dealt with in Part 3.

In part 2 of this study the object was to examine the anatomical aspects of fusion in the postnatal skeleton including sites and timings and sequence of fusions. The sites of fusion selected were those between postnatal skeletal elements rather than between multiple centres of ossification within bones, although the sternum was included. The major regions of fusion were all studied but a few minor sites e.g. uncinate processes to vertebral segments of ribs, entoglossal to rostral basibranchial and quadratojugal to jugal were excluded.

i. SKULL

INTRODUCTION

The marked degree of fusion found throughout the avian skeleton is reflected also in the skull where, although many sutures undergo eventual synostosis in the mammal also, this occurs relatively earlier in birds and so completely the sutures are often obliterated without subsequent trace, especially in carinates (de Beer 1937, Portmann 1950).

Attention has already been drawn to the difficulties of identifying individual cranial bones in the illustrations of most textbooks of avian anatomy so it follows that their articulations are equally difficult. Indeed the whole topic of arthology tends to receive scant attention in avian texts. In the 5th edition of Sisson and Grossman's Anatomy of the Domestic Animals there are chapters on syndesmology in all the sections devoted to the domestic mammals but none in the section on aves. Chamberlain's Atlas of Avian Anatomy although purporting to cover the fields of osteology, arthology and myology makes no attempt to indicate the location of any of the skull joints nor indeed any individual skull bones.

The embryological accounts of Hamilton (1952) and Romanoff (1960) show centres of ossification and approximate sites of articulation between them can be deduced, but without any great degree of accuracy.

The optimum time for the study of these joints would seem to be around the time of hatching when the bones have achieved their recognisable adult shape but are still clearly delineated. The diagrams of Jollie (1957) are particularly useful in this respect and much of the problem can be resolved from them. Doubt still remains about precise

eventual relationships around the fontanelles, which are large at this time, and also in the basis cranii. Additionally there are to be accounted for the articulations of the bone not described by Jollie (1957) and termed in this study the "orbitosphenoid".

The mandibular sutures are particularly poorly described perhaps due to the very complex relationships which the long splint like mandibular bones have with each other. Jollie's diagrams are again useful and are reproduced with some modification by Bellairs and Jenkin (1960) and Bellairs (1964), but the precise relationships and all the sites of articulation are still far from clear.

While there is general agreement on the eventual synostosis of the sutures and synchondroses of the neurocranium, the timing is variously described as occurring before the chick leaves the egg, (Bradley 1960) at an early age, (de Beer 1937), soon after hatching, (King and McLelland 1975), within the nestling stage (Bellairs and Jenkin 1960) and in the adult (Goodrich 1930).

Jollie (1957) describes the process generally as commencing in the basis cranii at about 75 days postnatal and spreading upward and forward until finally the frontal sutures close at 100 days.

In the mandible the sutures are described as undergoing obliteration which may obscure their identities (Bellairs and Jenkin 1960), while Parker (1869) lists particular sutures which can be traced in the adult as being those between the dentary and the angular and supraangular while all others close. Jollie (1957) describes the mandibular fusions as occurring between 75-90 days postnatal.

The fate of the symphysis mandibulae seems open to some doubt as Parker (1869) describes the dentaries as ankylosing early like the

premaxillae while Bellairs and Jenkin (1960) say that the 2 halves of the adult mandible fuse at the symphysis. Jollie (1957) describes the anterior centres of the dentaries as being fused across the midline almost from their first appearance.

It was decided to study skulls of birds at hatching to investigate fully and illustrate the various articulations formed in the neurocranium and mandible and to follow these through a series of growing birds to ascertain their fusion times. The neurocranium was taken to be that region which eventually forms the single fused mass obtainable by the maceration of adult specimens, and, therefore, excluded nasal, prefrontal and other members of the facial skeleton.

MATERIALS AND METHODS

The specimens used were those previously employed in the investigation of centres of ossification in the skull in part 1 of the study. The numbers involved, intervals of sampling and methods of preparation of specimens are summarised in table 2.

In following the various joints through the series, as far as possible every joint was identified on every specimen and recorded as "open" or "closed". Midline joints could not, of course, be studied on the sagittally sectioned specimens. Some articulations could be identified in only 1 of the 2 standard radiographic planes and certain joints could not be identified with certainty in either and were studied only in the alizarin specimens. These were those formed by the squamosal with the exoccipital, pleurosphenoid, prootic and supraoccipital, being joints which involved considerable overlap.

The radiographs were also found unsatisfactory for studying the

mandibular sutures which were, therefore, examined purely in the alizarin specimens.

The criterion for deciding that a joint was still "open" was a continuous line between adjacent bones, translucent on alizarin specimens or radiolucent on radiographs.

The range of time of fusion was calculated as extending from the last age at which fusion was invariably absent to the first age when it was invariably present. The median of the range was taken as the mean fusion time.

RESULTS

A. ARTICULATIONS

i. Neurocranium

The articulations formed by the bones of the neurocranium were as follows:-

1. Basioccipital Rostrally and rostroventrally with the parasphenoid/basisphenoid, laterally with prootic, caudolaterally with exoccipital (figs. 2, 63).
2. Exoccipital Rostrolaterally with squamosal, rostroventrally with the parasphenoid/basisphenoid (fig. 64), medially with the basioccipital, dorsally with the supraoccipital and on its deep aspect with the prootic (figs. 2, 63, 65).
3. Frontal Caudally with the parietal (fig. 66), ventrolaterally with the squamosal (fig. 67) and more rostrally and along the ventral margin of the orbital plate with the pleurosphenoid (figs. 64, 67). In the midline the frontal joined its fellow of the opposite side (fig. 66) and ventral to this union its rostral part joined the interorbital septum, ossified by the mesethmoid (fig. 4). The rostral tips of the frontals diverged to reveal the lamina dorsalis of the mesethmoid (fig. 5).

4. Mesethmoid Along its dorsal margin with the frontals (figs. 4, 5), ventrally with the parasphenoid/basisphenoid, rostrum (fig. 3) and at the ventral extremity of its caudal margin dorsal to the optic foramina with the orbitosphenoid (fig. 11).

5. Orbitosphenoid Rostrally and dorsally with the mesethmoid (figs. 11, 12), laterally with pleurosphenoid and medially with its fellow of the opposite side (figs. 10, 12).

6. Parasphenoid/basisphenoid As the separate centres forming this complex in the prenatal period had fused by the time of hatching, as discussed in part one, this was regarded as a single entity and no attempt made to describe which constituents formed its various articulations as it was felt that this could not be done in many cases with any great accuracy in these specimens. Superficially it articulated with the exoccipital caudally and the pleurosphenoid rostrally (figs. 64, 65), deeply with basioccipital caudally and prootic laterally (figs. 2, 63). The rostral prolongation, rostrum jointed the mesethmoid, accommodating it in a groove on its dorsal surface.

7. Parietal Ventrolaterally with the squamosal (figs. 1, 64), rostrally with the frontal (figs. 1, 66, 67), caudally with the supraoccipital and deeply with the prootic (figs. 2, 68) and at the midline with its fellow of the opposite side (fig. 66).

8. Pleurosphenoid Dorsally with the frontal and caudally with the squamosal (figs. 1, 64, 67). Ventrally with the parasphenoid/basisphenoid (figs. 2, 65) and medially with the orbitosphenoid (figs. 10, 12) and on its deep aspect with the rostralmost tip of the prootic (fig. 65).

9. Prootic The rostralmost tip joined the pleurosphenoid (figs. 1, 65), caudal to which it articulated with the deep surface of the parasphenoid/basisphenoid and more caudally, the basioccipital (figs. 2, 63).

Superiorly the prootic met the supraoccipital (epiotic part) from which it was at first widely separated but eventually fused to complete the otic capsule (fig. 68). Caudally it joined the exoccipital and laterally the parietal and more ventrally the squamosal (figs. 63, 68).

10. Squamosal Rostrally with pleurosphenoid, superiorly with the frontal rostrally and parietal more caudally, eventually for a short distance dorsocaudally with the supraoccipital across the previously existing lateral fontanelle and caudally with the exoccipital (figs. 1, 64, 67). Deeply it joined the prootic (fig. 63).

11. Supraoccipital Rostrally with the parietal (figs. 1, 66, 68), rostrolaterally with the squamosal for a short distance across the previously existing lateral fontanelle (figs. 64, 67) laterally with the exoccipital (figs. 1, 68) and deeply the epiotic part joined the superior projection of the prootic to complete the otic capsule (figs. 63, 68).

Thus a total of 27 articulations for the 11 bones constituting the neurocranium were identified, of which the majority were paired. They were:-

1. basioccipital - exoccipital*
2. basioccipital - parasphenoid/basisphenoid
3. basioccipital - prootic*
4. exoccipital - parasphenoid/basisphenoid*

*paired

5. exoccipital - prootic*
6. exoccipital - squamosal*
7. exoccipital - supraoccipital*
8. frontal - frontal
9. frontal - mesethmoid
10. frontal - parietal*
11. frontal - pleurosphenoid*
12. frontal - squamosal*
13. mesethmoid - orbitosphenoid*
14. mesethmoid - parasphenoid/basisphenoid
15. orbitosphenoid - orbitosphenoid
16. orbitosphenoid - pleurosphenoid*
17. parasphenoid/basisphenoid - pleurosphenoid*
18. parasphenoid/basisphenoid - prootic*
19. parietal - parietal
20. parietal - prootic*
21. parietal - squamosal*
22. parietal - supraoccipital*
23. pleurosphenoid - prootic*
24. pleurosphenoid - squamosal*
25. prootic - squamosal*
26. prootic - supraoccipital*
27. squamosal - supraoccipital*

*paired

ii. Mandible

As the articular and prearticular were fused together at the time of hatching they were treated as a single entity referred to as articular/prearticular. The dentaries were fused into 1 single structure so no mandibular synphysis was present.

The bones of the mandible were found to make the following articulations:-

1.	Angular	with articular/prearticular
		dentary
		splenial
		supraangular
		(figs. 7, 8, 69, 70)
2.	Articular/ prearticular	with angular
		supraangular
		(figs. 7, 8)
3.	Dentary	with angular
		splenial
		supraangular
		(figs. 7, 8, 69)
4.	Splenial	with angular
		dentary
		supraangular
		(figs. 8, 69)
5.	Supraangular	with angular
		articular/prearticular
		dentary
		splenial
		(figs. 7, 8, 69, 70)

Thus a total of 8 articulations for the mandibular bones were identified, all were paired.

They were:-

1. angular - articular/prearticular
2. angular - dentary
3. angular - splenial
4. angular - supraangular
5. articular/prearticular - supraangular
6. dentary - splenial
7. dentary - supraangular
8. splenial - supraangular

B.' FUSIONS

The ranges of times of fusion are shown in fig. 71 for the neurocranium and fig. 72 for the mandible, and the mean fusion times in fig. 73 for the neurocranium and fig. 74 for the mandible.

In the 23 neurocranial articulations examined by both methods the ranges obtained by each coincided to the extent that in 19 cases they were either overlapped or were contiguous. In 2 of the remainder the alizarin range was clearly earlier and the radiographic range was earlier in the remaining 2 cases. It was felt, therefore, that there was no evidence that either method tended to identify fusion earlier than the other.

The total ranges varied from 7 - 63 days. The greatest was in the case of the basioccipital - exoccipital where one alizarin specimen had shown fusion at 14 days postnatal. The next occurrence of fusion had been at 42 days in both alizarin specimens. This was a case, therefore,

when 1 specimen had increased the range by 28 days. It is interesting, however, that the range for this particular joint derived from the radiographic specimens was one of the widest also.

The articulation which had the earliest mean fusion time (basi-occipital - exoccipital) had the greatest range. The next greatest range was the parietal - prootic which occurred much later in the sequence while other relatively long ranges lay in the middle region. It was felt that there was no evidence that the position in the sequence of fusions influenced the range of fusion time.

The sequence of fusions in the neurocranium commenced around the basis cranii. The first 7 fusions involved the basioccipital, exoccipital or prootic then spread upward and forward to the supraoccipital, squamosal, pleurosphenoid and parasphenoid/basisphenoid. The frontal was first involved at 91 days (to pleurosphenoid) and the interfrontal closed at 102 days. The final fusions were the most rostral, involving orbitosphenoid and mesethmoid.

In the mandible the general direction appeared to be rostrally with the most caudal joint, prearticular to articular, being fused from the beginning. The suture between the splenial and the supraangular appeared to remain open being clearly distinct in the oldest specimens in the series. This does not mean that traces of others will not persist but all others did appear to undergo fusion.

The mean times of fusion varied from 39 - 119 days postnatal.

DISCUSSION

The alizarin and radiographic methods both yielded useful results. The alizarin method had the advantages of additional magnification available with the operating microscope and the ability to manipulate the

specimens to enable the lines of the joints to be followed. All were identifiable with this technique. The radiographic method was quicker both to perform and to examine all the individual joints on a large number of specimens. It gave very clear identification of many joints, the additional contrast obtained from the silver nitrate impregnation giving the bones sharply defined edges. It was not, however, found adequate to identify with certainty several of the joints of the squamosal where considerable degrees of overlap occurred and where the joint spaces seemed relatively narrow. The complex relationships of the long narrow mandibular bones meant that their articulations could only be satisfactorily worked out with the benefits peculiar to the alizarin method.

The sequence of fusion in the neurocranium agreed with that generally described by Jollie (1957) but the mean times of fusion were more widespread. Of the 27 articulations of the neurocranium 6 occurred below the lower limit of Jollie's range (75 days) and 9 were above his upper limit (100 days). All the lower ones were located in the basis cranii. 5 of the upper ones involved the orbitosphenoid or mesethmoid which were not included by Jollie.

The symphysis mandibulae being fused from time of hatching onwards appeared to confirm the view of Parker (1869) rather than some subsequent reports, though Jollie (1957) described it as being partially fused almost from the first appearance of the dentaries but also mentions a symphyseal ridge which becomes ossified across. This probably explains the confusion.

The theory that skull fusion is a necessary prerequisite for the spread of cranial pneumatisation has been advanced by several authors

but lack of details of the cranial fusion pattern has prevented its thorough investigation. The details yielded by this study were thought to provide a useful basis for a subsequent investigation on the development of cranial pneumatisation in the final part of this work.

ii. VERTEBRAL COLUMN

INTRODUCTION

Fusion of the centra, dorsal and ventral spines and transverse processes of vertebrae in the thoracic vertebral region produces the os notarium, a characteristic of several families of birds and occurring in the domestic fowl. There is general agreement that its composition is somewhat variable although there are few reports to provide details. Rydzewski (1935) described that of the domestic fowl as being more variable in composition than any other but containing 3 or 4 vertebrae from within the range C16 to T4. He described the degree of fusion also as being variable in that the free border of the dorsal spines could be fused but not the remainder. In the transverse plate were foramina which lay between the individual transverse processes and were filled with variable proportions of membrane and bone. The ventral processes could be fused partially or completely.

The synsacral fusion appears to be a regular finding in all birds. Reference has already been made to the uncertainty of the identity of the constituent elements. Additionally there is the problem of the variation in numbers of vertebrae involved, quoted ranges being from 14 - 16 in domestic fowl.

The pygostyle appears to be found regularly other than in tinamous

birds and most ratites (Bellairs and Jenkin 1960). The number of elements involved is again uncertain and figures quoted for domestic fowl usually range from 4 - 7.

The timing of these fusions appears to have received extremely scant attention. Harrison (1970) reports that fusion of bodies commences in the lumbosacral region at about 9 weeks postnatal but that in the thoracic region it does not occur until growth is complete. The pygostyle is sometimes said to be formed from a number of embryonic elements (George and Berger 1966, Feduccia 1975) but whether this means that these fuse in the prenatal period is not clear.

Between the fused thoracic and synsacral masses there are one or more free vertebrae usually thoracic no. 4. King and McLelland (1975) described this as the weak point in the overall design of the vertebral column, being particularly affected by disease notably spondylolisthesis or "kinky back", which occurs in broiler chickens. The condition was described by Wise (1970) and this included some brief anatomical details of the region.

The purpose of this section was to explore the pattern and timing of the various fusions and to provide further anatomical details of the 4th thoracic vertebra and its relations.

MATERIALS AND METHODS

Golden Comet pullets were used. 3 were sampled within 1 hour of hatching and further pairs of birds at 7 day intervals until 112 days, then at 14 day intervals until 182 days postnatal. From each bird portions of the vertebral column were removed from the second last cervical to the caudal end of the column. The penultimate cervical was

assumed to be C15 though this was not verified in this particular investigation. All specimens were treated with silver nitrate and radiographed dorsoventrally and laterally. This gave adequate detail to identify the outline of each vertebra including its processes as shown in fig. 75a - d. It should be noted that in this particular specimen the vertebrae from C13 caudally have been removed. Tracings were then made of all vertebrae on every radiograph including transverse processes on the dorsoventral plates and dorsal and ventral spinous processes on the laterals. This was found to assist greatly the identification of individual vertebrae after fusion had commenced when the situation became more complex. As in part I the junction between lumbosacral and coccygeal vertebrae was assumed to be at the first recognisably wider intercentral gap in the belief that this indicated the caudal limit of the future synsacrum.

A portion of the vertebral column from C16 to the middle of the synsacrum was removed from 2 birds aged 63 days and similarly from 2 adult birds. These specimens were first radiographed laterally and dorsoventrally. Their permitted movements were examined and they were then dissected for the examination of ligaments. The area adjacent to T4 was finally sectioned midsagittally, decalcified and prepared for histological examination with staining by haematoxylin and eosin.

RESULTS

A. Notarium

The intervals between centra, transverse processes and dorsal and ventral spines were inspected in all vertebrae from C15 to T4 and all fusions recorded. The range of time of fusion and mean fusion time for

each joint were calculated as before. Ranges of fusion times are shown in fig. 76a - d, and mean fusion times in fig. 77.

No fusion was found in birds up to 105 days postnatal. All birds examined thereafter showed fusion between the dorsal spines C16-T1, T1-2 and all except 2 between T2-3. These latter 2 birds were at the upper end of the scale and it was thought that this condition would probably have remained permanently. In one case T3 was completely unfused to T2 and this appeared to be an instance of a 3 boned notarium. In the other the centra of T2 and T3 were fused and the transverse processes slightly fused. This appeared to be a 4 boned notarium with element T3 only partially fused.

The intercentral joints commenced their fusion a little later and those between T1-2 and T2-3 were found slightly in advance of C16-T1.

The intertransverse fusions occurred latest of all and considerable variation was encountered in the degree of fusion between the processes. Any slight fusion was recorded as positive and all cases were very symmetrical bilaterally.

By far the greatest variability was found in fusion between the ventral spines. This was found only between C16-T1 and T1-2 and then varied greatly in extent. The inconstancy of occurrence meant that it was not possible to define the upper limit of the range with certainty in the limited numbers available and therefore only the lower limit is indicated in fig. 76d.

B. Synsacrum

The numbers of "synsacral" vertebrae in the 45 specimens in this series were calculated. Synsacral was taken to include T5 and all lumbosacral vertebrae as defined under materials and methods and the

results are shown in table 13.

Number	Frequency
14	2(4.4%)
15	26(57.7%)
16	17(37.7%)

Table 13 Numbers of synsacral vertebrae found in 45 Golden Comet pullets.

Inspection of adult macerated specimens indicated that fusion occurred between all dorsal spines, centra and most transverse processes but costal processes though fused to transverse processes to variable extents did not fuse to each other, remaining distinct.

The ranges of times of fusion and mean fusion times are shown for each joint calculated as for the notarium in figs. 76 and 77.

No fusions were found up to 49 days postnatal but were found to commence thereafter in intercentral joints from LS2-8 and interspinous joints LS1-8. The intercentral fusion spread gradually caudally to reach most caudal joints by about 162 days postnatal. Regardless of numbers of synsacral vertebrae in any individual the most caudal joint was always unfused throughout the period of investigation. The intercentral joint T5-LS1 was found to fuse about 133 days postnatal.

The interspinous fusion also spread gradually caudally reaching the most caudal members of the series towards the end of the period of investigation but did not involve the last vertebra. Fusion was found between T5 and LS1 about 109 days postnatal.

Intertransverse fusion commenced later in the region LS3-10 from about 102 days postnatal onwards. The most caudal fusion found was between LS10-11 and variable fusion in the region T5-LS3 was found in the latter part of the period of investigation.

C. Pygostyle

The total numbers of coccygeal vertebrae in 43 specimens in the series are shown in Table 14.

Number	Frequency
7	16(37.2%)
8	19(44.2%)
9	8(18.6%)

Table 14 Numbers of coccygeal vertebrae in 43 Golden Comet pullets.

The variation in pattern and timing of fusion was far greater here than in the preceding regions. Fusion was seen in 1 bird at hatching where 3 elements were involved and in a few in the neonatal period. The oldest bird without any fusion was 49 days postnatal. Up to 105 days postnatal many birds only showed fusion of the arches and dorsal spines with the separate centra clearly visible but after that age the centra were always involved also. Fusion was often so complete that it was difficult to determine accurately the number of elements which had coalesced particularly as transverse processes were not developed. In particularly difficult cases it was found necessary to approximate the number involved by comparing the size of the fused pygostyle with the

size of the adjacent free elements.

The number of fused vertebrae appeared to vary from 2 - 4. Even in the older birds in the series several had only 2 involved with others closely related as if fusion with these might yet occur.

D. Articulations of the 4th thoracic vertebra

The joint spaces of T3-4 and T4-5 were clearly visible on the radiographs of the adult birds. On dorsoventral view the joint surfaces appeared markedly concavoconvex being concave cranially. No trace of the adjacent intercentral joints could be found, complete fusion having occurred. The radiographs of the 63 day old birds showed that all the intercentral joints in the region were unfused but the spaces of the T4 joints were considerably wider than the others.

When the permitted movements were examined it was found that in the adult specimens only lateral flexion was possible at these joints. In the 63 day old birds slight movement was detected at the other interthoracic joints but the vast majority of movement occurred at the joints of T4 and was principally lateral flexion though a small amount of dorsal and ventral flexion was also appreciable.

In all specimens the ventral longitudinal ligament was examined. This was a fine, though distinct, structure passing continuously over the vertebrae and particularly in the adults was seen to spread into the intervening joint capsules of the T4 joints. The dorsal longitudinal ligament did not exist as a distinct structure but rather as strands of fibres bridging between the width of adjacent centra and merging with the joint capsules.

No conjugate ligament was present, the rib heads having migrated caudad in birds to articulate with a single facet on the corresponding

centrum.

Histologically the joints of the adult specimens had the appearance of typical synovial joints. The surfaces were lined by hyaline articular cartilage deep to which was a thin layer of compact bone enclosing pneumatised trabecular bone (fig. 78)

In the growing birds a fibrocartilage disc was present which was found completely united with the articular cartilages of the centra of T2 and T3 (fig. 80) but was in the process of separating from these cartilages in the joints of T4 to adopt the form of an intraarticular meniscus interposed between 2 synovial cavities as shown in fig. 79. No pneumatisation was present in these vertebrae.

DISCUSSION

The curious os notarium, peculiar to certain families of birds would appear to be a modification for stability and rigidity as discussed in the general introduction to this part of the study. Its general design, cross-shaped on transverse section is very like the angle girder of engineering, this being a structure renowned for its strength. Yet the notarium is not an invariable finding in birds and is, of course, not formed in the young as premature fusion would prevent further growth within that region. The notarium becomes extensively pneumatised as will be described later but presumably this does not seriously weaken the structure.

The number of elements involved and the location within the vertebral column agreed with the findings of Rydzewski (1935).

Fusion proceeded from 105 days onwards, earlier than reported by Harrison (1970) who described it as occurring only after growth was

complete at about 20 weeks in the broiler birds in his study.

The number of elements in the developed synsacrum ranged from 14-16 agreeing with most reports. This certainly included the last thoracic but how many could be regarded as coccygeal remains uncertain. In the literature there seem to be 2 schools of thought, one taking the view that 1 coccygeal is incorporated into the synsacrum and the other believing there to be several. As seen in part 1 there are no obvious morphological differences in the centres of ossification of the last synsacral element and those immediately cranial to it and the free coccygeal elements caudal to it. Yet in one way this last vertebra behaved differently in that it remained unfused from the remainder until at least the end of the investigation. The last thoracic was also rather later in fusing into the synsacral mass than the first few lumbosacral elements immediately caudal to it.

The commencement of synsacral fusion about 53 days postnatal was marginally earlier than quoted by Harrison (1970) who first detected it at 9 weeks in his broiler birds.

The total number of coccygeals varied from 7-9, agreeing with the results in part 1 but being rather less than most reports suggest (usually 11-13) but this generally includes 1 or more in the synsacrum. The numbers of free coccygeals, about 5, agree but the number fused into the pygostyle, even allowing for the difficulty of assessing the number accurately in some instances would appear to be about 2-4 rather than 5-6 or more as is usually claimed.

A few interesting anatomical points have been shown regarding the articulations of vertebra T4, but it would be dangerous to attempt to explain the condition of spondylolisthesis on the basis of these,

particularly as according to Wise (1970) this is purely a condition affecting broiler birds and these investigations were carried out on laying birds.

Two differences from the mammalian pattern found in the intercentral joints of T4 are that they are purely synovial in the adult and that the longitudinal ligaments are very poorly developed. The lack of an intervertebral disc also removes one of the main stabilising ligaments of the joint. It may be that the widespread synostosis cranial and caudal to these joints has led to a great reduction in the longitudinal ligaments as they are unnecessary in these regions and consequently this may have caused them to be underdeveloped over the surviving moveable joints.

In contrast with the adult arrangement the 63 day old birds had the remains of the fibrocartilaginous disc characteristic of avian intercentral joints, (Newton and Gadow 1896, Harrison 1970). However, at this stage these were not typical secondary cartilaginous joints but had joint cavities developing with the fibrocartilage disc taking the form of a meniscus whereas the adjacent intercentral joints were typical secondary cartilaginous joints. It could be that failure of the disc to undergo separation from the articular cartilages in the process of development of the synovial joints might lead to severe stresses in other parts of the vertebrae which could cause subluxation or fracture.

One potential cause of weakness which was not present in the 63 day old birds was pneumatisation but this was found extensively in adults. This topic will be pursued further in part 3.

iii. STERNUM

INTRODUCTION

As referred to in part one, disagreement existed on the centres of ossification in the sternum until the relatively recent alizarin studies. Early reports on fusion were therefore disregarded in this instance and it was found that there was very little recent information on the subject of fusion times in the sternum. Hamilton (1952) claims that the centres fuse together about the 3rd month.

It was felt that neither examination of adult macerated sterna nor alizarin specimens of chicks at hatching gave a precise picture of how the centres eventually fuse, and it was decided to investigate this and the timing of the various fusions.

MATERIALS AND METHODS

Golden Comet pullets were used. 3 were killed at hatching and then in groups of 2 at 7 day intervals until 112 days postnatal, and at 14 days until 182 days postnatal.

The sterna of all were removed, treated with silver nitrate and radiographed dorsoventrally. They were then sectioned in the midsagittal plane and each half radiographed laterally.

The outlines of the centres were traced from each radiograph and the joints formed and occurrence of fusion in each joint recorded.

The range of time of fusion for each joint and the mean fusion times were calculated as before.

RESULTS/

RESULTS

A. Articulations

There were 3 paired articulations.

1. Craniolateral process - caudolateral process
2. Body - craniolateral process
3. Body - caudolateral process

These are indicated in fig. 81.

B. Fusions

The range of time of fusion for each of the 3 joints is shown in fig. 82, and the mean fusion times are shown in fig. 83.

In each case after fusion was first detected it was found in every instance thereafter. The fusion between the craniolateral process and caudolateral process occurred slightly in advance of the other two.

DISCUSSION

The fusion of these joints occurs relatively early in the total growth period of the sternum. All 3 fusions are in a fairly central location within the sternum and further bone growth can occur, therefore, at the peripheral ends of the processes and at the cranial and caudal extremities of the body and its ventral projection, the carina or keel. Indeed the caudal end of the keel was found to continue to grow until towards the end of the period of the investigation.

In view of the considerable economic importance of this region of the skeleton and its related musculature it is surprising to find so little recent data available on the question of fusion. Comparison with

the situation in broiler birds would be a useful extension particularly in view of the fact that time of fusion was so constant in the laying birds used in this study.

iv. OS COXAE

INTRODUCTION

When a macerated specimen from an adult domestic fowl is examined the general configuration of the constituent elements, Ilium, Ischium and pubis can be deduced when compared to the separate centres seen in the chick at hatching, though the marked degree of fusion so characteristic of birds normally removes all vestige of the sites of fusion. These can, therefore, only be deduced approximately and particularly doubtful are the precise relationships around the acetabulum. Harrison (1975) has described the wall of the acetabulum as being formed half by the ilium, a quarter by ischium and a sixth by the pubis.

Pubic and probably ischial symphyses occurred in Archaeopteryx (Heilmann 1926) but they are absent in the domestic fowl as in most neornithes but do occur in different forms in ostrich and rhea.

The constituent bones are said to be fused in the adult (Kaupp 1918, Bellairs and Jenkin 1960) but very few details on fusion times and sequence can be found. Harrison (1975) described it as commencing in the acetabular region in the 3rd month, when the pubis and ischium fused, and being completed during the 4th month.

This section set out to check the sites of fusion, to indicate these and to investigate their fusion pattern.

MATERIALS AND METHODS/

MATERIALS AND METHODS

Golden Comet pullets were used, 3 were killed within an hour of hatching and further groups of 2 at 7 day intervals to 112 days then at 14 day intervals till 182 days.

In each specimen the left and right os coxae were removed until this became impossible due to extensive synostosis to the vertebral column. At this stage the synsacrum was sawn midsagittally. Lateral radiographs of all specimens were taken.

The outlines of ilium, ischium and pubis were traced and the occurrence of fusion in each was recorded.

The range of time of fusion for each joint and the mean times of fusion were calculated as before.

RESULTS

A. ARTICULATIONS

5 articulations were recognised as shown in fig. 84.

The ilium articulated with the ischium at 2 sites, the cranial being in the wall of the acetabulum and the caudal being behind the ischiatic foramen which became therefore surrounded by ilium and ischium.

The ilium joined the pubis in the wall of the acetabulum.

The ischium and the pubis also articulated at 2 sites, the cranial being again in the acetabular wall and the caudal being behind the obturator foramen, which became, therefore, surrounded by ischium and pubis.

B. FUSIONS

The range of time of fusion for each of the 5 joints is shown in fig. 82 and the mean fusion times in fig. 83.

In 4 joints it was found that once fusion was first detected it was present in all cases examined subsequently. The exception was the caudal joint between ischium and pubis, which was the last to occur and showed slight variability.

DISCUSSION

The mean fusion times in the wall of the acetabulum agreed with the general times quoted by Harrison (1975).

The pattern is not unlike that of the sternum in that the fusions are located fairly centrally within the whole mass, and occur relatively early in the total growing period which continued almost throughout the investigation. Certainly the fusions had all occurred before adulthood although previous reports have inferred otherwise.

v. CARPUS AND METACARPUS

INTRODUCTION

Although it is well known that the distal carpal elements in birds become fused to the metacarpals to form the carpometacarpus their subsequent identification was not found possible in examination of adult macerated specimens, due again to the completeness of fusion obscuring the constituent elements. Neither did examination of specimens showing recently developed centres precisely clarify where the various sites of fusion would eventually occur.

This is another region of the bird skeleton where intense prenatal interest has not been matched by postnatal investigations, and very little could be found concerning timing and sequence of the various fusions.

Various authors such as Newton and Gadow (1895) have stated that the distal row of carpals fuse to the metacarpals in embryo. This refers to the mesenchymal anlagen and within this fused mass develop the chondral and later osseous centres as detailed by Montagna (1945) and Holmgren (1955). However these statements have led authors like McLeod et al (1964) to make misleading remarks like "the distal carpal bones fuse with the metacarpals early in development", clearly implying osseous fusion at this stage. Bradley (1960) and others confine themselves to comments so general that they are misleading such as "the metacarpus in the adult is in the form of a single bone".

The most detailed information available appears to be in the work of Schinz and Zangerl (1937) who deal with several postnatal topics including this and show a series of drawings of alizarin specimens of known ages enabling the sequence of development to be followed, but the ages chosen do not provide great detail on the question of individual fusions. Their findings will be discussed along with those from this study.

MATERIALS AND METHODS

This investigation was performed on 2 groups of birds. Firstly White Leghorn birds were sampled in pairs, 1 male, 1 female at hatching and at 7 day intervals till 119 days and the final male bird at 126 days. Secondly groups of 3 Golden Comet females were sampled at hatching and at 7 day intervals till 112 days and at 14 day intervals till 182 days. Lateral radiographs of the left carpus and manus were taken. Birds of up to 21 days were first treated with silver nitrate to increase their skeletal density. Serial tracings of all the centres of carpus and

metacarpus from all birds were made. The various sites of fusions were identified and the occurrence of fusion in each was recorded. Range of time of fusion for each site and mean fusion times were calculated as before.

RESULTS

A. ARTICULATIONS

7 joints which underwent subsequent fusion were identified and are shown in fig. 85.

These were:-

1. distal carpal III - metacarpal II
2. distal carpal III - metacarpal III
3. distal carpal IV - metacarpal III
4. distal carpal IV - metacarpal IV
5. metacarpal II - metacarpal III
6. metacarpal III - metacarpal IV (proximal)
7. metacarpal III - metacarpal IV (distal)

B. FUSION

The range of time of fusion for each joint is shown in fig. 86 for Golden Comet females, White Leghorn females and White Leghorn males and all birds collectively. The mean times of fusion are similarly shown in fig. 87.

In all 3 groups it was found that the first fusion to occur was that between distal carpal III and metacarpal II. The remaining fusions were all found at about the same time. In one group (White Leghorn males) that between distal carpal III and metacarpal III was slightly earlier

and in the Golden Comet females the fusion between metacarpal II and metcarpal III was slightly later.

The earliest mean fusion time was at 53 days, distal carpal III - metacarpal II in White Leghorn females, all others occurred before 95 days. In many cases the times coincided for the 3 groups.

The ranges of time of fusion were minimal. In most instances it was the case that once fusion was first detected it was found in all cases thereafter. Slight variation occurred in the fusion between metacarpal II and metacarpal III.

DISCUSSION

Examination of the sites of fusion in the series of developmental stages reveals the interesting fact that there is no fusion directly between the distal carpal elements III and IV. Indeed from the time of appearance of their ossification centres distal IV is considerably further distal. This cannot be deduced from examination of adult fused specimens. Montagna (1945) described and illustrated the forces generated by the growth of radius, ulna, large metacarpals and centrale I and considered that this causes a shift in position of the carpal elements, flattens them, destroys their identity and forces their fusion. The shift in position of distal carpal IV would appear to assist the adoption of the position of ulnar deviation at the carpus.

The times of fusion of each joint showed little variation in each group and indeed there was little difference between the groups and no evidence that one group tended to undergo fusion earlier than the others.

All of the fusions occurred close together on the time scale, many coinciding. All occurred well before the end of the growth period as a

whole and from examination of the tracings of the birds at time of fusion and at the end of the period of investigation it was thought that there was no evidence of further linear growth of carpals or metacarpals after fusion had occurred.

Comparison of these results with those of Schinz and Zangerl (1937) found agreement on the first occurrence of fusion being between distal carpal III and metacarpal II but this was claimed to occur rather later, between 86 and 96 days postnatal. All other fusions were indicated to occur after 96 days postnatal but as the next stage shown was the adult no further details of timing and sequence were available. Thus in this study the fusions in all groups except 1 had occurred by 91 days postnatal while in Schinz and Zangerl's (1937) study most had yet to occur after 96 days postnatal.

vi. TARSUS

INTRODUCTION

The tarsal region of the fowl is a site of some importance in disease being involved in a variety of conditions generally referred to as perosis or "slipped tendon" which have assumed rather greater importance with intensive methods of husbandry. Probably for this reason more attention has been paid to this area than most others. Although there are some disagreements between these existing reports it did appear that the further investigations to be carried out in this study could be usefully compared to the previous results to give further information on differences attributable to breed or sex.

The work of Latimer (1927) was mainly a growth study of the long

bones in White Leghorns, males and females. The method was gross examination and it is not at all clear what criteria were used to assess the completion of growth due to the confusion of the term epiphysis as already discussed. As the work is frequently quoted it was decided to include its results on completion of growth at distal end of tibia and proximal end of metatarsus for comparison.

The tarsus was another postnatal topic dealt with by Schinz and Zangerl (1937). Here the method was alizarin staining and a useful series of drawings were given of stages at 10 day intervals but only up to 75 days postnatal after which only the adult is shown.

Bruce et al (1946) provided a useful radiographic study on cockerels as a basis for investigation of disease conditions. They appear to have been unaware of the work of Schinz and Zangerl (1937) dealing specifically with this topic and quote Olsson (1936) and Nielson (1942) as the only previous sources of reference on the subject. Both contain many obvious inaccuracies and their results have not been included here for comparison. The study by Bruce et al (1946) was on live birds and gives a series of radiographs variously selected from the 10 birds in the study. The stages were selected to show morphological changes rather than fusions so these cannot be timed very precisely. Perhaps due to its publication in a pathology journal this particular paper has not been quoted in subsequent anatomical work on the subject.

Church and Johnson (1964) performed another radiographic study in broiler birds following up Latimer's (1927) work but dealing more clearly with the fusion of the tarsal elements though terming them epiphyses. Although male and female birds were included no mention is made of sex differences in fusion times.

Franceschini (1967) has been discussed at some length already, being divergent in view regarding the number of proximal tarsal elements. The timing of the fusions of these was given but the distal row of tarsal elements was not included.

In this final section on fusion it was intended to demonstrate the sites of fusion, to investigate their timing and examine evidence of variation between the 3 groups available and previous reports.

MATERIALS AND METHODS

Radiographic studies were made on 2 groups of birds. White Leghorns were killed in pairs, 1 male and 1 female at hatching and at 7 day intervals until 119 days postnatal and the final male bird at 126 days. Golden Comet pullets were sampled in groups of 3 at hatching and at 7 day intervals till 112 days then at 14 day intervals till 182 days. Birds up to 21 days postnatal were first treated with silver nitrate to increase their skeletal density.

Bruce et al (1946) commented on the danger of identifying fusion between the tibial diaphysis and proximal row of tarsal elements on craniocaudal radiographs and, therefore, in addition to these lateral radiographs were taken. Indeed the only real value of the craniocaudal view proved to be in the identification between the proximal tarsal elements themselves and after this had occurred only lateral plates were taken of the Golden Comet birds.

Serial tracings of all radiographs were made and used to confirm the sites of fusion and to record the occurrence of fusion in them.

The ranges of times of fusion and mean fusion times were calculated as before.

RESULTS

A. Sites of fusion

3 sites were identified:-

1) Proximal medial - proximal lateral tarsal as shown in fig. 88a. This proved to be the first fusion to occur and the 2 remaining sites were as shown in fig. 88b.

2) Proximal tarsal - tibial diaphysis

3) Distal tarsal - metatarsals II - IV.

B. Occurrence of fusion

The ranges of times of fusion are shown for Golden Comet females, White Leghorn females and White Leghorn males and all birds collectively in fig. 89. Mean fusion times are shown in fig. 90 similarly.

The sequence of the 3 fusions was the same in all 3 groups, the first fusion to occur being between the proximal medial and proximal lateral tarsals. This was found to have an earlier mean fusion time in the White Leghorn females than the males but was latest in the Golden Comet females.

The remaining 2 fusions were both found to have the same mean fusion time in each of the 3 groups and in fact both had the same range in each group though there were differences among the 3 groups. In all White Leghorn birds they were in fact both similarly present or absent in every individual. This was not the case in the Golden Comets as shown in table 15.

Fusion	Bird No.	Days Postnatal 84	91	98	105	112
Prox. Tarsal - Tibial diaph.	1	-	+	-	-	+
	2	-	+	+	+	+
	3	-	+	+	+	+
Dist. Tarsal - Metatarsals II-IV	1	-	+	+	+	+
	2	-	-	-	-	+
	3	-	+	+	+	+

\+ Fusion present

\- Fusion absent

Table 15 Occurrence of fusion in tarsus of Golden Comet pullets.

This demonstrates that 3 birds, numbers 91/2, 98/2, 105/2 showed fusion at the proximal site but not at the distal whereas 2 birds, numbers 98/1 and 105/1 showed fusion at the distal site but not at the proximal. However the range of time of fusion of both was 84 - 112 days postnatal and the mean fusion time was 98 days postnatal for each.

In these 2 fusions the mean fusion times were again earlier in the White Leghorn females than in the White Leghorn males, but was in this case earliest of all in the Golden Comets.

DISCUSSION

The results from this study are shown compared with those in previous reports in table 16.

Author	Type of Bird	Sex	Method	Prox.Medial Tarsal - Prox.Lat. Tarsal (days p/n)	Prox.Tarsal - Tibial Diaph. (days p/n)	Dist.Tarsal - Metatarsals II-IV (days p/n)
Latimer (1927)	White Leghorn	F	Gross	-	114	139
	White Leghorn	M	Gross	-	158	195
Schinz & Zangerl (1937)	?	?	Alizarin	55-65	65-75	Over 75
Bruce et al. (1946)	Rhode Island Red/ light Sussex	M	x-ray	70-77	77-98	119-140
Church & Johnson (1964)	New Hampshire/ Barred Rock	M & F	x-ray	70	98	112
Franceschini (1967)	?	?	Serial Section Histo.	70-77	150	-
DAH	Golden Comet	F	x-ray	56-70	84-112	84-112
DAH	White Leghorn	F	x-ray	42-49	98-105	98-105
DAH	White Leghorn	M	x-ray	42-63	112-119	112-119

Table 16 Comparison of quoted ranges of times of fusion in tarsus.

The earlier occurrence of fusion at all 3 sites in the White Leghorn females than in males agrees with the findings of Latimer (1927). Among others only Church and Johnson (1964) specify that both sexes were included in their study but they do not mention any sex differences in fusion times.

There was broad agreement in the quoted fusion times for the proximal medial and proximal lateral tarsals. The lower limits of the ranges found for the White Leghorn birds in this study are lower than in any previous. The overall mean fusion time of 56 days was slightly earlier than all previous claims. The highest upper limit of ranges were 77 days quoted by Bruce et al. (1946) for cockerels and by Franceschini (1967) for unspecified birds. There are great variations in the quoted figures for the remaining 2 fusions. In the case of the proximal tarsal to tibial diaphysis these varied from 158 days postnatal (Latimer 1927) to only 65-75 days postnatal (Schinz and Zangerl 1937). No doubt there are considerable genuine differences in time of completion of long bone growth depending on genetic and environmental factors but as referred to already there is some doubt as to the accuracy of Latimer's method and his times particularly 195 days postnatal for the distal tarsal to metatarsal II - IV fusion do seem rather late particularly as both he and Church and Johnson (1964) say that the general growth period ends about 20 weeks postnatal. The figures quoted by Schinz and Zangerl (1937) on the other hand seem rather low particularly as they are not much later than the figures for the fusion between the 2 proximal tarsal elements. The results for these 2 fusions in this study lay somewhere in the middle of the quoted range.

Perhaps the most interesting result from this investigation is that

the ranges of time of fusion and mean fusion times were identical for the fusions between proximal tarsal and tibial diaphysis and distal tarsal and metatarsal II - IV in all 3 groups, whereas in all previous reports the distal fusion was claimed to occur later than the proximal. Consequently the ranges obtained in this study for the distal fusion are rather earlier than in most other accounts discounting Schinz and Zangerl (1937) where the series did not extend far enough to localise this fusion.

The completion of these tarsal fusions does not necessarily mean that growth in length of tibiotarsus and tarsometatarsus is at an end. In the case of the former Latimer (1927) and Church and Johnson (1964) both claim that growth continues longer at the proximal end of the tibia than the distal. On the other hand Church and Johnson (1964) say that the distal end of metatarsus ceases to grow first and Latimer (1927) says that there appears to be no "distal epiphysis" in the metatarsus which seems to mean that he claims there is no growth at all at this end.

As in the carpus and metacarpus there were no clearly marked differences in mean fusion times in the Golden Comets and in the White Leghorns. These are fairly similar types of bird and further comparison with broiler birds would be particularly useful.

CONCLUSIONS FROM PART 2 OF STUDY

The sites of fusion in the postnatal skeleton have been identified and illustrated. The sequence of fusion in each selected region and the mean fusion time and range of fusion time of each joint have been calculated and shown for the Golden Comets.

When it was possible to compare results obtained by alizarin red S staining with those obtained by radiography close agreement was found.

Limited comparisons made with White Leghorn female birds did not reveal any marked differences in mean fusion times in carpus, metacarpus or tarsus.

In the tarsus there appeared to be rather earlier fusion in White Leghorn females than in males but this was not conclusive in the carpus and metacarpus.

The intercentral joints formed by the 4th thoracic vertebra were initially secondary cartilaginous joints similar to the adjacent joints in the series. While these underwent subsequent synostosis those of the 4th thoracic transformed to synovial joints having little ligamentous support as no intraarticular joint disc remained, the longitudinal ligaments were poorly developed and the conjugate ligament was absent.

PART THREE

PNEUMATISATION IN THE POSTNATAL SKELETON

GENERAL INTRODUCTION

Widespread pneumatisation of the skeleton is another outstanding characteristic of birds (Evans 1969). Its existence has been known for a very long time, its discovery being attributed to Emperor Frederick II in the 13th century (Goodrich 1930 and King 1957), yet many functional and anatomical details remain unknown today.

Although pneumatisation is believed to have occurred in the pterosaurian reptilian ancestors of birds and in dinosaurs it is thought to have been absent in the earliest birds, Archaeopteryx and Hesperonis (Goodrich 1930). Pneumatisation is not a finding exclusive to birds as it also occurs in parts of the skulls of the crocodiles and in mammals in the form of paranasal sinuses and the mastoid air cells of man and certain other species, but the widespread occurrence in many parts of the skeleton is a truly avian peculiarity.

As used here the term pneumatisation refers to the presence of an air-filled cavity within a bone. It does not include other air-filled cavities which are merely surrounded by bone such as the nasal meatuses. Descriptions of pneumatisation of the head including nasal fossae, subocular diverticula and dilatations of the nasolacrimal duct have led to confusion in this respect, having been subsequently regarded as being accounts of skull pneumatisation.

The function of the widespread pneumatisation of the skeleton has been the subject of much debate particularly on account of its considerable variation in extent between species. The small ornithological text by Headley (1895) gives an interesting and informative review dealing with several of the paradoxes of the situation. It forms the basis of many subsequent reviews although is rarely credited as being the

source of the information. The following is based largely on Headley's account.

Many small birds which are very good fliers are relatively or completely apneumatic while most big, strong-flying birds are well pneumatised. In these large birds the pneumatised bones have a relatively greater diameter which produces relatively greater strength against bending stresses from the strong muscles attaching to them. The strength is increased in spite of the fact that the cortex is not proportionally increased in its thickness. The pneumatisation of such bones causes a greater degree of reduction in mass than in relatively small bones. The presences of internal strutting like the Warren truss pattern used in aircraft design is further evidence of strengthening in bones of this type as first shown by Prochnow and subsequently reproduced by Thompson (1942), Young (1950), Parker and Haswell (1962) and others. The net result of these various modifications is bones of increased strength unaccompanied by proportional increase in mass.

Various authors have taken the view that the loss in body weight produced by replacement of the marrow by air is too small to be of significance, indeed Goodrich (1930) emphasised this by saying that the difference is less than that resulting from feeding. However it may be that reduction in bone mass is a modification to <u>assist</u> in the ability to fly though not of great effectual magnitude. It would appear that any way of keeping down the overall body weight is of value in this respect as the largest flying birds weigh only up to about 35 lb. whereas flightless birds have weights of up to several hundredweights.

The presence of pneumatisation in flightless species is also mentioned by Headley as occurring in the Kiwi (skull only) but being widespread in

the Ostrich, Emu, Rhea and Cassowary. This has been used to argue against the development of pneumatisation as a modification for flight. Yet as it is now generally agreed that the flightless ratites are descended from ~~carinates~~ flying ancestors it may be that the presence of pneumatisation has lingered on though the ability to fly has been lost due to the great increase in body weight and other factors.

Headley also remarked on the great reduction in extent of pneumatisation in diving species and advanced the view that this prevented too great buoyancy in these birds. Harrison (1958) developed this by detailing the changes in volume caused by underwater pressure at depths to which ducks commonly dive i.e. up to 60 feet.

Other suggested explanations of avian pneumatisation are that it serves to increase the available area of respiratory mucous membrane for moistening and controlling the temperature of inspired air (Verheyen 1953, Bellairs and Jenkin 1960) or that in the case of the skull it provides insulation against heat loss which may be of particular advantage when flying at altitude and may assist in dissemination of shock waves on impact (Verheyen 1953).

Despite the long standing interest in the subject and the large number of reports describing the variation in extent of pneumatisation between species there are few reliable accounts concerning the domestic fowl. King (1957) reviewed the literature on the subject and performed a reinvestigation the results of which largely confirmed the findings of Campana (1875). Both excluded the skull from study and in both the numbers involved were small although in a subsequent investigation King and Kelly (1956) examined the 3rd thoracic vertebra and sternal ribs on a larger number of specimens. Campana (1875) believed that in the adult,

or by breeding age, the boundary between medullary and pneumatic parts of the bones has already been achieved and is fixed for each species independently of mode of life though he admitted that under unspecified physiological or pathological conditions the proportions may vary a little. King (1957) on the other hand found considerable variations in the 6 birds in his series.

There are many reports on skull pneumatisation in wild species, particularly in relation to its final extent in the roof of the cranium and the timing of its development. The fowl skull has been studied by Bremer (1940a) and some mention is made by Jollie (1957). Both are concerned principally with the routes of communication to the pneumatised spaces of the skull bones rather than in details of which bones are actually pneumatised and to what extent.

There is very little information available on the timing of the whole process in the fowl. Bradley (1960) described it as a late development and Headley (1895) claimed that all bones were solid in the young, while Bremer (1940a) has described prenatal involvement of skull bones.

In the 3rd part of the study it was proposed to investigate the distribution of pneumatisation in the adult skull, to survey the variability in extent of pneumatisation in the whole adult skeleton and to follow the timing of development of the process in the postnatal skeleton.

1. THE PNEUMATISED BONES OF THE ADULT SKULL

INTRODUCTION

Although a well recognised phenomenon, pneumatisation of the skull receives only the most general of descriptions in most avian textbooks.

Portmann (1950) makes the general statement that the neurocranium and splanchnocranium are pneumatised from the tympanic cavity and the dermatocranium from the nasal cavity. Harrison (1964) quotes the skull as an area where pneumatisation is diminished in diving species, woodpeckers which use their skulls as a hammer and swift flying birds such as the galliforms. The lack of detailed information on pneumatisation of specific bones is probably again accounted for by the completeness of fusion of the skull in the adult with the consequent difficulty in identification of the constituent elements. George and Berger (1966) give some more details for birds in general. They list the otic capsule, occipital, parietal, frontal, quadrate and interorbital septum as being involved. The dermatocranium has been studied in many species by ornithologists to investigate the extent and timing of development of pneumatisation as a means of assessing age and for functional significance as discussed in the general introduction. The standard method in these investigations has been the transillumination of macerated skulls whereby opaque areas are regarded as indicating pneumatisation. The diagrams shown are only of the roof of the cranium but indicate that even in species regarded as incompletely pneumatised the frontals, parietals and supraoccipital are involved. Of the large number of species studied the vast majority have been described as completely or incompletely pneumatised and very few as non-pneumatised though this condition has been claimed by Harrison (1957b) in diving ducks and cormorants. In these ornithological reports there are no other anatomical details of the base or sides of the cranium or of the facial skeleton, pterygoid, quadrate or mandible. The diagrams given have to be interpreted with some caution as nomenclature is sometimes unconventional such as the term nasal bone

being applied to the frontal.

Information specific to the domestic fowl is limited to one major account by Bremer (1940a), subsequently quoted by a number of authors, and a few other isolated statements by other workers.

Bremer (1940a) described the development of 2 air filled cavity systems, the first being the subocular sacs formed by epithelial outgrowths from the middle meatus of the nasal cavity and a dilatation of the naso-lacrimal duct. These air filled sacs become partially surrounded by bones secondarily but were not regarded as true pneumatisation as they are not contained within bone. However, subsequent authors such as George and Berger (1966) have on the basis of this work described the nasal region as being pneumatised by the epithelial outgrowths of the nasolacrimal duct and from nasal cavity which could readily be taken to mean pneumatisation of bone. The second air sac system described by Bremer (1940a) was derived from the tympanic cavity and involved true pneumatisation of bone. It occurred in one of 2 ways either by direct expansion into the surrounding bones dorsorostrally, dorsally and caudally to give partial pneumatisation of the region or from 2 tubular outgrowths from the tympanic cavity which pneumatise the more distal bones of the cranium and face. In the former case no further anatomical detail of the bones involved is given. In the latter the more dorsal tubular outgrowth caused complete pneumatisation of the quadrate and extended from there to the pterygoid and many bones of the maxillary region though these are not specified or shown. The ventral outgrowth was described as passing near the quadrate but not normally entering it and reaching the mandible which was said to be normally unpneumatised in the fowl and most other species. Subsequently this outgrowth degenerated.

Coues (1890) described a pneumatic foramen on the back of the articular surface of the mandible for the entrance of air, when any, which seems to imply variable occurrence of pneumatisation.

Jollie (1957) in his account of skull development in the fowl mentions pneumatisation of the supraoccipital and parietal bones from the middle ear cavity and of the quadrate from the inner ear cavity which seems likely to be an error for the middle ear cavity.

De Beer (1937) gave no description of pneumatisation of the domestic fowl's skull but refers to the situation in the pigeon (Columba livea), implying the situation to be similar. The description was said to be based on the work of Strasser (1905) and Lurje (1906). Specific regions pneumatised by the tympanic cavity were quoted as interorbital septum, basal plate, periotic capsule, parietal, frontal, squamosal, occipital, quadrate and articular leading to Meckel's cartilage. A similar process from the nasal cavity was said to involve the maxilla, premaxilla and lacrimal. Indeed the only exceptions to pneumatisation were said to be the jugal and quadratojugal. If this information is accurate and applicable to the fowl it would seem to indicate a more extensive involvement than described by Bremer (1940a) although he did not list the bones so specifically.

This study set out to look at pneumatisation in the adult skull by identifying its occurrence in the various osseous elements already described, and to provide illustrations of the appearance of pneumatised bone which has not previously been done.

MATERIALS AND METHODS/

MATERIALS AND METHODS

8 adult birds 5 hens and 3 cockerels of unknown breed were examined. 2 were injected with a mixture of Indian Ink and gelatin via a 30 gauge needle inserted alternately into the left and right auditory tubes via the choana. The remaining birds were examined without prior injection. The heads were removed, frozen and sawn midsagittally with a fine band-saw. The bones of the neurocranium thus sectioned were supraoccipital, parietal, frontal, orbitosphenoid, parasphenoid/basisphenoid and basi-occipital. The approximate boundaries of these bones were defined with reference to figs. 1 and 2. If the section had missed the midline mesethmoid forming the interorbital septum this was sectioned with for-ceps. The 2 half mandibles, pterygoids, quadrates and the facial skeleton bones, premaxilla, maxilla, prefrontal, jugal, quadratojugal, palatine and nasal which were all readily identifiable were removed and sectioned. The 2 halves of the neurocranium were then sectioned in 2 transverse planes, the first immediately caudal to the orbit through the postorbital process and the second immediately caudal to the external acoustic meatus. Reference to figs. 1 and 2 shows that the first section passed through frontal, squamosal, pleurosphenoid and parasphenoid/basisphenoid and the second through parietal, exoccipital, parasphenoid/basisphenoid, prootic and basioccipital. All sectioned surfaces were examined under a Zeiss operating microscope.

Portions of the facial bones, mesethmoid, quadrate, pterygoid, mandible and columella were taken from 4 birds and prepared for histo-logical examination with staining by haematoxylin and eosin.

RESULTS/

RESULTS

1. Appearance of pneumatised bone

By gross examination the pneumatised spaces were found to occur in cancellous bone forming the diploe of the flat bones of the roof and in the chondrocranium. The appearance was of a honeycomb-like network of spheroidal cavities lined by a smooth reflective membrane. Pneumatised regions could readily be distinguished from adjacent marrow filled trabecular spaces and the thin cortical bone. The gross appearance of various pneumatised cranial bones is shown in figs. 91-99.

Histologically the appearance was of cavities either empty or containing pale staining amorphous material and lined by a thin epithelium of squamous cells with a lamina propria which was very thin other than where the numerous blood vessels intervened. These pneumatised spaces were readily distinguishable from the marrow filled cavities. The histological appearance of various pneumatised bones is shown in figs. 100-103.

2. Occurrence of pneumatisation

The occurrence of pneumatisation in birds examined grossly is shown in table 17, and histologically in table 18. A summary of the occurrence of pneumatisation in the bones examined is given in table 19.

Bird No.	P1	P2	P3	P4	P5	P6	P7	P8
Sex	0_x	0_x	0_x	0_x	0_x	0_x	0_x	0_x
Injection with Indian ink	+	+	-	-	-	-	-	-
Premaxilla	-	-	-	-	-	-	-	-
Nasal	-	-	-	-	-	-	-	-
Maxilla	-	-	-	-	-	-	-	-
Jugal	-	-	-	-	-	-	-	-
Quadrato-jugal	-	-	-	-	-	-	-	-
Palatine	-	-	-	-	-	-	-	-
Mesethmoid	+	+	+	+	+	+	+	+
Prefrontal	-	-	-	-	-	-	-	-
Frontal	+	+	+	+	+	+	+	+
Parietal	+	+	+	+	+	+	+	+
Supraoccipital	+	+	+	+	+	+	+	+
Basioccipital	+	+	+	+	+	+	+	+
Exoccipital	+	+	+	+	+	+	+	+
Basisphenoid/ Parasphenoid	+	+	+	+	+	+	+	+
Prootic	+	+	+	+	+	+	+	+
Squamosal	+	+	+	+	+	+	+	+
Orbitosphenoid	+	+	+	+	+	+	+	+
Pleurosphenoid	+	+	+	+	+	+	+	+
Quadrate	+	+	+	+	+	+	+	+
Pterygoid	+	+	+	+	+	+	+	+
Mandible	-	-	-	+	+	-	-	+

\+ Pneumatisation present
\- Pneumatisation absent

Table 17 Bones of adult skull showing pneumatisation.

I Gross examination.

Bird No.	P2	P3	P4	P5
Sex	0_x	0_x	♀	♀
Premaxilla	-	-	-	-
Nasal	-	-	-	-
Maxilla	-	-	-	-
Jugal	-	-	-	-
Quadratojugal	-	-	-	-
Palatine	-	-	-	-
Mesethmoid	+	+	+	+
Prefrontal	-	+	-	-
Quadrate	+	+	+	+
Pterygoid	+	+	+	+
Mandible	-	-	+	+
Columella	-	-	-	-

+ Pneumatisation present - Pneumatisation absent

Table 18 Bones of adult skull showing pneumatisation.
II Histological examination.

Pneumatisation constant	Pneumatisation variable	Pneumatisation absent
Frontal		Premaxilla
Parietal	Prefrontal	Nasal
Supraoccipital	Mandible	Maxilla
		Jugal
Basioccipital		Quadratojugal
Exoccipital		Palatine
Prootic		Columella
Basisphenoid/Parasphenoid		
Mesethmoid		
Squamosal		
Orbitosphenoid		
Pleurosphenoid		
Quadrate		
Pterygoid		

Table 19 Summary of occurrence of pneumatisation in bones of adult skull.

All the bones forming the neurocranium were constantly pneumatised as was the mesethmoid. This does not mean that all these were fully pneumatised but that evidence of pneumatisation was found within them.

The quadrate and pterygoid were the only other bones constantly pneumatised.

Of the 2 bones found variably to be pneumatised the mandible was found to be so in 3 cases out of 8 examined grossly. The histological examination confirmed the gross results. In all cases pneumatisation was found in the most proximal part in the region of the articular bone. The prefrontal was found pneumatised in 1 bird only by histological examination the condition in that bird having been missed by gross examination.

No other bones of the facial skeleton showed evidence of pneumatisation nor did the columella.

DISCUSSION

In the 2 birds which were injected traces of the injection medium were found in the base of the cranium and parts of the side wall and roof. Extensive leakage occurred from the tympanic cavity and in neither case did the injected material enter the quadrate, pterygoid or mandible. Examination showed the extent of penetration to be far from complete and indeed it filled only the more obviously pneumatised areas which could be readily identified without prior injection. This confirmed the view of King and Kelly (1956) and King (1957) that pneumatised can accurately be distinguished from non-pneumatised bone without injection. As it was not the intention to investigate further communications of these pneumatised spaces the technique of prior injection was discontinued.

In 1 instance histological examination revealed pneumatisation that had not been observed by gross inspection. This was in a prefrontal bone which is a particularly thin plate of bone. With this exception complete agreement was found between the 2 methods. It was felt, therefore, that for the next stage of the work, which was a survey of the variability in extent of pneumatisation in the cranium in a large number of birds, gross examination alone without prior injection would provide sufficiently accurate results.

The lack of pneumatisation of the facial skeleton appeared to contradict Bremer (1940a) and certainly contradicted de Beer (1937) though he referred to Columba livea. The pneumatisation of the neurocranium, quadrate and pterygoid agreed with Bremer (1940a) and others. The variable existence of pneumatisation in the mandible was not in accordance with the description of Bremer (1940a) although he did in fact suggest that the mandible might on occasion be pneumatised by saying that "normally" in the fowl and most other species pneumatisation is absent. The same hint is found in the description of the mandible by Coues (1890).

ii. EXTENT OF PNEUMATISATION IN THE ADULT

INTRODUCTION

Reference has already been made to the considerable ornithological interest in pneumatisation of the dermatocranium. The variations in the adult extent have been studied in many species by Verheyen (1953), Harrison (1961) and others. Winkler (1972) in a similar study on the Herring Gull (Larus argentatus) claimed a tendency towards greater pneumatisation in males. The method in these investigations has consistently been transillumination of the macerated skull and the recording of the transparent

windows as the areas lacking pneumatisation. The domestic fowl has not been studied by this method though Harrison (1957) described the galliform birds collectively as incompletely pneumatised, being "swift fliers".

There are very few other sources of information on extent of cranial pneumatisation. Bignon (1887) claimed that generally in birds the base of the cranium, the occipital, the quadrate and the articular part of the mandible were highly pneumatised. Bremer (1940a) in his study on development of cranial pneumatisation in the fowl said virtually nothing on extent, though he described the quadrate as being eventually completely pneumatised. His statements concerning the mandible have been discussed already.

The pneumatisation of the post cranial skeleton in the fowl was investigated by King (1957) and King and Kelly (1956). The previous literature was extensively reviewed and the extreme diversity of opinion ranging from pneumatisation in the humerus only (Hunter 1774) to pneumatisation in all bones (Booth 1939) was discussed. In discussing the results of these studies the nomenclature has been converted to correspond with that employed in the present study to avoid confusion when the results are subsequently compared. The results indicated that the distribution of pneumatisation was as follows:-

1. Pneumatisation constant

- Vertebrae

  All cervical other than the atlas and axis.

  Thoracic 4, 5.

  Lumbosacral mass - cranial 1/3.

- Humerus

- Coracoid (though diagram suggests there may have been absence in 1 instance).

2. Pneumatisation variable

Vertebrae

Thoracic 1, 2, 3.

Lumbosacral mass - caudal 2/3.

Pelvic girdle.

Ribs

Vertebral 1 and 2.

Sternal 2 and 3.

Sternum.

3. Pneumatisation absent

Vertebrae

Atlas, axis.

Free coccygeal and pygostyle.

Ribs

Vertebral 3 - 7.

Sternal 1, 4, 5.

Scapula.

Clavicle.

All wing bones except humerus.

All pelvic limb bones.

These results largely agreed with those of the only other comprehensive study of the topic, that of Campana (1875). Small differences were that Campana (1875) implied that sternal ribs 1, 4 and 5 belonged to group 2, thoracic vertebrae 1, 2, 3 to group 1 and the coracoid to group 2.

The claims of Campana (1875) regarding lack of variation in a given species in extent of pneumatisation have been mentioned already. Some

variations were described such as an old cockerel in which the left coracoid was slightly pneumatised and the right not at all. King (1957) included 6 birds in the main part of his study and great variations in extent of pneumatisation were noted, particularly in the pelvic girdle, sternum and coracoid. Considerable asymmetry was encountered. The cervical vertebrae and humerus were consistently fully pneumatised.

In the study of pneumatisation of the notarium King and Kelly (1956) concluded that in adults pneumatisation was more extensive in males than females but that sexual activity in the female was not a factor. Other possible factors were discussed by King (1957) though the small number of specimens and lack of information on their origin did not permit the significance of these factors to be explored.

This study was undertaken to see if these previous results of occurrence of pneumatisation would be confirmed on a larger number of specimens, and to obtain further and more precise details on variability in extent of pneumatisation in a group of birds where many potential sources of variation due to differences in age, housing, management and breed were removed. It was felt this would give valuable information on the degree of variation attributable to purely individual genetic differences.

MATERIALS AND METHODS

51 adult Golden Comet birds aged $2\frac{1}{2}$ years, 48 hens and 3 cockerels were used. These were breeding stock originally from the same hatch and had always been kept under the same conditions of management.

They were killed with pentobarbitone sodium and examined without prior injection. The bones to be examined were stripped of flesh,

frozen, and sectioned by a fine bandsaw. This gave a cleaner cut surface. After sectioning they were examined by naked eye and under the operating microscope as necessary.

The skull was sectioned midsagittally and the bones forming the cut surface examined as previously. The left half of the mandible, the left quadrate and the left pterygoid were sectioned with forceps. The occurrence and extent of pneumatisation in the frontal, parietal, supraoccipital, basiocciptal, parasphenoid/basisphenoid, pterygoids and quadrates were recorded. Extent referred to the distance which pneumatisation had spread through each bone rather than the completeness of pneumatisation in the bone. This proved of particular importance in the dermatocranium as will be discussed later. The articular region of the mandible, the orbitosphenoid and the mesethmoid were simply recorded as pneumatised or not being too small to readily permit application of gradations.

Thereafter the skull bones were macerated and the 2 halves of the neurocranium apposed and transilluminated. The outline of the dermatocranium and transparent fenestrae were drawn.

The whole vertebral column when frozen could readily be sawn midsagittally thus enabling the individual vertebrae to be counted and identified. The occurrence and extent of pneumatisation in cervicals and thoracics were recorded. The synsacral vertebrae were counted by reference to ridges along the roof of the vertebral canal or by the intervertebral foramina. As before the synsacrum was considered to consist of the last thoracic and the lumbosacral vertebrae as there was no obvious indication of which might represent coccygeals. As these vertebrae were somewhat smaller they were recorded simply as pneumatised

or not.

The vertebral and sternal ribs were counted and sectioned and recorded as pneumatised or not.

The sternum was sawn midsagittally through the body and keel and the pneumatised length expressed as a percentage of the ossified length. The processes were sectioned with forceps.

The 2 halves of the pelvic girdle were sawn longitudinally through ilium and ischium, and evidence of pneumatisation recorded. The pubis was sectioned with forceps.

The coracoids, humeri and femora were sawn longitudinally and pneumatised length expressed as a percentage of total length measured to the surfaces of articular cartilages. The remaining bones distal to the humerus and femur were not examined as it was felt they were no longer regarded as controversial.

RESULTS

A. Pneumatisation in skeletal regions

i. Skull

The occurrence and extent in the bones examined by section are shown in tables 20 and 21 for the hens and tables 22 and 23 for the 3 cockerels.

Bone	++++ (100%)	+++ (75%)	++ (50%)	+ (25%)	- (0%)	Total No.
Frontal	4	42	2	0	0	48
Parietal	46	0	1	1	0	48
Supra-occipital	47	0	1	0	0	48
Basioccipital	0	1	16	31	0	48
Parasphenoid/ Basisphenoid	48	0	0	0	0	48
Quadrate	44	3	1	0	0	48
Pterygoid	41	0	0	1	6	48

Table 20 Occurrence and extent of pneumatisation in cranial bones - hens.

Bone	+	-	Total
Orbitosphenoid	48	0	48
Mesethmoid	48	0	48
L. Mandible (articular region)	22	26	48

+ Pneumatisation present

- Pneumatisation absent

Table 21 Occurrence of pneumatisation in 3 cranial bones - hens.

Bone	C1	C2	C3
Frontal	+++	++++	+++
Parietal	++++	++++	++++
Supraoccipital	++++	++++	++++
Basioccipital	++++	++++	++++
Parasphenoid/ Basisphenoid	++++	++++	++++
L. Quadrate	++++	++++	++++
L. Pterygoid	++++	++++	++++

++++ 100%)
+++ 75%) Pneumatisation present

Table 22 Degree of pneumatisation present in cranial bones - cockerels.

Bone	C1	C2	C3
Orbitosphenoid	+	+	+
Mesethmoid	+	+	+
L. Mandible	+	+	+

+ Pneumatisation present.

Table 23 Occurrence of pneumatisation in 3 cranial bones - cockerels.

All the neurocranial bones examined were pneumatised in all birds.

In the roof of the cranium the frontal was seldom recorded as fully pneumatised but mostly as three quarters. As this referred purely to the extent it gave no direct information about the completeness of replacement of marrow, and often considerable amounts persisted (fig. 94). The region lacking in pneumatisation was always at the caudal extremity of the bone towards the articulation with the parietal. If the section had been made in a slightly paramedian plane an area was seen where diploe was lacking, the bone being a single solid plate with no pneumatisation (fig. 104). The parietal and supraoccipital were pneumatised throughout in virtually all specimens (fig. 93).

In the basis cranii the parasphenoid/basisphenoid was always pneumatised throughout though traces of red marrow were always present (fig. 91). On the other hand the basioccipital was never found fully pneumatised in the hens and in only one case was recorded as more than half pneumatised, the remainder being made up of cancellous bone containing marrow and compact bone forming the median portion of the occipital condyle. In all 3 cockerels, however, the basioccipital was fully pneumatised, only a thin shell of bone persisting beneath the articular cartilage of the condyle (fig. 92).

In the splanchnocranial bones, pneumatisation was always present and usually complete in the quadrate but was absent from the pterygoid in 6 hens. When present in the pterygoid it was virtually always complete. In the 22 cases where pneumatisation of the mandible occurred this was always confined to the region of the articular bone.

The stages of pneumatisation of the roof of the cranium as assessed by transillumination are shown in fig. 105 and the frequency of occurrence

of each stage in the 51 birds in the series is shown in table 24.

Stage of cranial pneumatisation	No. of birds	% Age
1	2	3.9
2	6	11.8
3	11	21.6
4	7	13.7
5	22	43.1
6	3	5.9

5 birds showed asymmetric extent.

These birds are recorded on basis of left side.

Table 24 Extent of pneumatisation in roof of neurocranium of 51 birds, assessed by transillumination.

The stages ranged up to complete (stage 6 found in 3 birds). All except stage 1 suggested pneumatisation was present throughout the whole of the midsagittal plane. Very little asymmetry was found and this never exceeded 1 stage.

ii. Vertebral column

Cervical region

The numerical variations found are shown in table 25.

No. of cervical vertebrae	Frequency	
15	1	(2.0%)
16	48	(94.1%)
17	2	(3.9%)

Table 25 Numerical variation in cervical vertebrae.

The pattern of pneumatisation of the cervical vertebrae is shown in fig. 106 for hens and the data for the 3 cockerels is shown in table 26.

Cervical Vertebrae	C1	C2	C3
1	-	-	-
2	-	-	-
3	++	-	+++
4	+++	-	+++
5	+++	+++	+++
6	+++	+++	+++
7	+++	+++	+++
8	+++	+++	+++
9	+++	+++	+++
10	+++	+++	+++
11	+++	+++	+++
12	+++	+++	+++
13	+++	+++	+++
14	+++	+++	+++
15	+++	+++	+++
16	+++	0	+++

+++	100%)	
+++	75%)	Pneumatisation present
++	50%)	
+	25%)	
-		Pneumatisation absent
0		Bone absent

Table 26 Degree of pneumatisation in cervical vertebrae - cockerels.

The atlas was never pneumatised and consisted entirely of compact bone.

The axis was pneumatised in 2 hens, completely in one and 75% in the other (figs. 107 and 108).

The 3rd cervical showed a slightly lower incidence of pneumatisation than succeeding members of the series, cervicals 4 - 9 which were always pneumatised. C10 - 16 or 17 were pneumatised in the vast majority of birds.

In most vertebrae where pneumatisation occurred it was complete. In all instances except 1 it involved at least half of the vertebra.

Thoracic region

No numberical variation was encountered.

The pattern of pneumatisation in the hens is shown in fig. 109 and the data for the 3 cockerels is given in table 27.

Thoracic vertebrae	C1	C2	C3
1	++++	++++	++++
2	++++	-	-
3	++++	-	-
4	++++	++++	++++
5	++++	++++	++++

++++ 100% pneumatisation present

\- pneumatisation absent

Table 27 Occurrence of pneumatisation in thoracic vertebrae - cockerels.

In no vertebra was pneumatisation constantly present or absent. The bones most frequently pneumatised were T1 and T4 and the least frequently was T3. Pneumatisation tended to be complete in all except T2 where it did not extend more than half way through the bone in 18 out of the 32 birds involved.

Lumbosacral region

The numerical variations encountered are shown in table 28.

No. of Lumbosacral vertebrae	No. of birds	% Age
15	45	88.2
14	6	11.8

Table 28 Numerical variation in lumbosacral vertebrae.

The pattern of pneumatisation is shown for the hens in fig. 110 and the data for the 3 cockerels is given in table 29.

Lumbosacral Vertebrae	C1	C2	C3
1	+	+	+
2	+	+	+
3	+	+	+
4	+	+	+
5	+	+	+
6	+	+	+
7	+	+	+
8	+	-	-
9	+	-	-
10	+	-	-
11	+	-	-
12	+	-	-
13	+	-	-
14	+	-	-
15	+	-	-

\+ Pneumatisation present.

\- Pneumatisation absent.

Table 29 Occurrence of pneumatisation in lumbosacral vertebrae - cockerels.

No vertebra showed pneumatisation constantly present or constantly absent. In the cranial third of the group (lumbosacrals 1-5) the incidence was noticeably greater than in succeeding regions. The middle third (lumbosacrals 6-10) showed a decreasing incidence in a caudal direction and the caudal third (lumbosacrals 11-14 or 15) showed a fairly even level of occurrence, being the lowest in the region.

Coccygeal region

Numerical variations encountered in the free coccygeals are shown in table 30.

No. of free coccygeal vertebrae	No. of birds	% Age
5	37	72.5
4	14	27.5

Table 30 Numerical variation in free coccygeal vertebrae.

No pneumatisation was detected.

iii. Ribs

Vertebral

No numerical variation was found.

The occurrence of pneumatisation in the hens is shown in table 31.

Vertebral Rib No.	Pneumatisation +ve			Pneumatisation -ve	Total
	Unilateral	Bilateral	Total		
1	8	25	33	15	48
2	8	5	13	35	48
3	1	0	1	47	48
4	0	0	0	48	48
5	0	0	0	48	48
6	0	0	0	48	48
7	0	0	0	48	48

Table 31 Occurrence of pneumatisation in vertebral ribs - hens.

It was found to occur in ribs 1-3 only and was found to be most common by far in the 1st. Indeed in the 3rd it was found in only 1 bird and then unilaterally.

In the 3 cockerels the first rib only was bilaterally pneumatised in all 3.

Sternal

The numerical variation found is shown in table 32.

No. of pairs of sternal ribs	No. of birds	% Age
5	49	96.1
4	2	3.9

Table 32 Numerical variation in sternal ribs.

No pneumatisation was detected.

iv. Sternum

The occurrence of pneumatisation in regions of the sternum is shown in table 33 for hens and table 34 for the 3 cockerels.

Sternum	Pneumatised	Non-pneumatised	Total No.
Body & keel	20	28	48
Craniolateral Process	0	48	48
Caudolateral Process	0	48	48

Table 33 Occurrence of pneumatisation in sternum - hens.

Sternum	C1	C2	C3	
Body & keel	+	+	+	+ pneumatisation present - pneumatisation absent
Craniolateral Process	+ (bilat.)	+ (bilat.)	-	
Caudolateral Process	+ (unilat.)	-	-	

Table 34 Occurrence of pneumatisation in sternum - cockerels.

In the hens the only region where pneumatisation was found was the body and keel but 2 cockerels were pneumatised in the craniolateral and in the proximal part of the caudolateral processes. These cockerels were also pneumatised in the body and keel.

The pattern of extent of pneumatisation in body and keel in the hens

is shown in fig. 111. It never exceeded 40% of the sternal length and was always confined to the most cranial part (fig. 112).

v. Os coxae

The occurrence of pneumatisation in the hens is shown in table 35.

Bone	Pneumatised	Non-pneumatised	Total
Ilium	2 (Bilat.) 2 (Unilat.)	44	48
Ischium	0	48	48
Pubis	0	48	48

Table 35 Occurrence of pneumatisation in os coxae - hens.

It was found in 4 birds only and was confined to a small area of the ilium. None was found in the 3 cockerels.

vi. Humerus

The occurrence of pneumatisation in the hens is shown in table 36 and the pattern of variation in extent in fig. 113.

Pneumatisation		+ve	Pneumatisation	Total
Unilateral	Bilateral	Total	-ve	
14 (6 left) (8 right)	23	37	11	48

Table 36 Occurrence of pneumatisation in the humerus - hens.

The 3 cockerels were bilaterally pneumatised and the extents are shown in table 37.

Humerus	C1	C2	C3
Left	98	80	53
Right	98	87	78

Table 37 Percentage extent of pneumatisation. The humerus - cockerels.

11 hens showed no pneumatisation of the humerus at all and in 14 it was confined to 1 side as shown in fig.114. Therefore, only about 50% of birds were bilaterally pneumatised. The extent of pneumatisation was found to vary from 10 - 100% although even in the birds where it extended throughout the length of the bone, traces of red marrow still persisted. Only 13 bones (11 from hens and 2 from cockerels) belonged to this group. In all cases where it was incomplete it was found at the proximal extremity of the bone.

vii. Coracoid

The occurrence of the pneumatisation in the hens is shown in table 38 and the pattern of variation in extent in fig. 115.

Pneumatisation		+ve	Pneumatisation	Total
Unilateral	Bilateral	Total	-ve	
5 (2 left) (3 right)	5	10	38	48

Table 38 Occurrence of pneumatisation in coracoid - hens.

The data for the 3 cockerels is shown in table 39.

Coracoid	C1	C2	C3
Left	85	55	0
Right	94	82	0

Table 39 Percentage extent in pneumatisation of the coracoid - cockerels.

Only 12 birds (10 hens and 2 cockerels) showed any pneumatisation at all and this was unilateral in approximately half of these. The extent varied up to 40% of the length and was always confined to the sternal end of the bone.

viii. Femur

No evidence of pneumatisation was found.

Summary of occurrence of pneumatisation in the adult skeleton

The occurrence of pneumatisation in the skeletons of all 51 birds in the study is summarised in fig. 116, indicating bones in which pneumatisation was constant, variable or absent.

B. Pneumatisation in individuals

1. A total pneumatisation score was calculated for each bird. Maximum possible scores were:-

Cranial air sacs

Frontal	4
Parietal	4
Supraoccipital	4
Basioccipital	4
Orbitosphenoid	1
Parasphenoid/ basisphenoid	4
Mesethmoid	1
Quadrate	4
Pterygoid	4
Mandible	1
TOTAL	31

Cervical air sac

Cervical vertebra	2	4
	3	4
	4	4
	5	4
	6	4
	7	4
	8	4
	9	4
	10	4
	11	4
	12	4
	13	4

Cervical air sac (cont'd)

Cervical vertebra	14	4
	15	4
	16	4
Thoracic vertebra	1	4
	2	4
	3	4
Vertebral rib	L1	1
	R1	1
	L2	1
	R2	1
	L3	1
	R3	1
TOTAL		78

Clavicular air sac

L. Humerus	10
R. Humerus	10
L. Coracoid	10
R. Coracoid	10
Sternum - body & keel	10
processes	4
TOTAL	54

Abdominal air sacs

Thoracic vertebra	4	4
	5	4
Lumbosacral vertebra	1	1
	2	1
	3	1
	4	1
	5	1
	6	1
	7	1
	8	1
	9	1
	10	1
	11	1
	12	1
	13	1
	14	1
	15	1
Ilium	L	1
	R	1
TOTAL		25

Maximum possible total pneumatisation score 188

The distribution of total pneumatisation scores is shown in fig. 117 indicating hens and cockerels separately. For the hens the mean was 112.9 with standard deviation 13.95.

2. Correlation of extent of pneumatisation

a) Within an air sac group

The bones pneumatised by the clavicular sac were selected as their extent of pneumatisation could be most accurately assessed. Correlation between pneumatised bones were sought using the Pearson r and Spearman rank order correlation coefficient, r_{rho}. The results are shown in tables 40 and 41.

x	y	n.	r.	P.	Correlation
L. coracoid	R. coracoid	7	0.940	$P < 0.01$	+
L. humerus	R. humerus	26	0.310	$P > 0.20$	-
L. humerus	L. coracoid	7	0.583	$P > 0.05$	-
R. humerus	R. coracoid	7	0.687	$P < 0.01$	+
Sternum	coracoids	13	0.325	$P > 0.20$	-
Sternum	humeri	33	0.089	$P > 0.20$	-

Table 40 Clavicular sac bones - Pearson r.

x	y	n.	r_{rho}	P.	Correlation
L. coracoid	R. coracoid	7	0.960	$P < 0.01$	+
L. humerus	R. humerus	26	0.423	$P < 0.05$	+
L. humerus	L. coracoid	7	0.848	$P < 0.05$	+
R. humerus	R. coracoid	7	0.929	$P = 0.01$	+
Sternum	coracoids	13	0.321	$P > 0.1$	-
Sternum	humeri	33	0.072	$P > 0.1$	-

Table 41 Clavicular sac bones - r_{rho}.

b) Between air sac groups

Pneumatisation scores for the bones in the cranial, cervical clavicular and abdominal sac groups were calculated and correlations sought using the Pearson r and Spearman rank order correlation coefficient, r_{rho}. The results are shown in tables 42 and 43.

x	y	n	r	P	Correlation
Cranial	Cervical	51	-0.256	$P > 0.20$	-
Cranial	Clavicular	51	0.480	$P < 0.001$	+
Cranial	Abdominals	51	-0.023	$P > 0.20$	-
Cervical	Clavicular	51	0.049	$P > 0.20$	-
Cervical	Abdominals	51	0.378	$P < 0.01$	+
Clavicular	Abdominals	51	0.178	$P > 0.20$	-

Table 42 Air sac groups - Pearson r.

x	y	n	r_{rho}	P	Correlation
Cranial	Cervical	51	-0.146	$P > 0.20$	-
Cranial	Clavicular	51	0.291	$P < 0.05$	+
Cranial	Abdominals	51	0.052	$P > 0.20$	-
Cervical	Clavicular	51	0.071	$P > 0.20$	-
Cervical	Abdominals	51	0.279	$P < 0.05$	+
Clavicular	Abdominals	51	0.069	$P > 0.20$	-

Table 43 Air sac groups - r_{rho}.

3. Correlation of total and regional pneumatisation

The Pearson r for the total pneumatisation score and individual air sac group pneumatisation scores were calculated.

x	y	r
Total	Cranial	0.999
Total	Cervical	0.860
Total	Clavicular	0.746
Total	Abdominals	0.601

Table 44 Total pneumatisation and regional pneumatisation - Pearson r.

DISCUSSION

Compared with other regions of the skeleton the skull showed relatively little variation in pneumatisation both in occurrence and extent. This was particularly true of the basis cranii but rather less true of the frontal, where, though always present it varied in degree and was occasionally found complete both by section and transillumination. This did not, therefore, agree with Harrison (1957) for galliform birds in general.

Transillumination of most skulls suggested that pneumatisation was complete in the midline but this was not confirmed for the frontal bone by section. The area of solid bone seen in the paramedian sections was found to be translucent and it is felt, therefore, that the fenestrae revealed by transillumination indicated areas where the diploe has not developed. The opaque areas would appear to indicate the presence of diploe and only if pneumatisation extended throughout would this provide an accurate measurement.

The mandible was again found to be variably pneumatised as in the preliminary study on the skull and clearly contradicts the view of Bremer (1940a). The quadrate was always found pneumatised but occasionally the pterygoid was not. This situation is in keeping with the description of spread of pneumatisation occurring from the quadrate to the pterygoid via the joint capsule (Bremer 1940a). It would therefore be necessary to have pneumatisation of the quadrate before the pterygoid could be involved and any failure to achieve the maximum extent of pneumatisation would first involve the pterygoid.

In the postcranial skeleton the numerical variations encountered were similar to those in the previous parts of the study.

When the results of this study concerning occurrence of pneumatisation in the postcranial skeleton are compared with those of King (1957) and King and Kelly (1956) the following differences emerge. The only bones in this study found constantly pneumatised were cervical vertebrae 5-9, all others in their first group being found variably pneumatised.

The second group, those variably pneumatised, was considerably greater in this study and included additionally the axis, and all other vertebrae except the atlas and cervicals 5-9, and coccygeals the humerus, coracoid and vertebral rib 3, but excluded sternal ribs 2 and 3.

The bones not pneumatised agreed subject to the addition of sternal ribs 2 and 3 and the deletion of the axis and vertebral rib 3.

King (1957) commented that although his list agreed closely with the findings of Campana (1875) it was rather more limited than suggested by most textbooks. The results of this study show fewer regularly pneumatised bones still. There may be 2 reasons for this. The first is that in a process like pneumatisation, where considerable variation occurs between individuals, examination of a large number of specimens, as in this study, will inevitably reveal more variation than a small sample up to a limit where all variation has been revealed. It should not be assumed that this has been achieved in this study. Further variation might well have been revealed had more similar birds been available. The most suspect bones are cervical vertebrae 5-9 and the quadrate could well have fallen into the variable group along with the pterygoid and mandible. This would leave only the neurocranium and it seems unlikely on account of the completeness of pneumatisation, especially in the basis cranii, that variation would occur here.

The second possible explanation would be that these birds as a

group are relatively lightly pneumatised. The relative lack of pneumatisation in the humerus is probably the most surprising result in this whole study in view of the previous widespread agreement on its pneumatisation. The coracoid and os coxae show a much lower incidence than implied by Campana (1875) and King (1957). All sternal ribs in this study were negative compared with a 10% incidence in a similar number of birds examined by King and Kelly (1956).

In examining the extent of pneumatisation within individual bones it was found that even in cases of full pneumatisation in basis cranii, humerus or vertebrae traces of red marrow always persisted as described by Campana (1875).

The extent in certain bones was rather less than indicated by previous authors as has been mentioned already, particularly in os coxae, sternum, humerus and coracoid.

The cervical air sac pneumatises the cervical, thoracic vertebrae 1 - 3 and vertebral ribs (King 1975). It appears that this grows cranially and caudally to reach these bones and generally reaches the 3rd cervical in the cranial direction. It would seem, therefore, that if the sac succeeds in reaching the axis it can give rise to pneumatisation in it. The structure of the axis is similar to the succeeding cervical vertebrae, containing cancellous bone unlike the atlas which is all compact bone. As only 2 cases of pneumatisation of the axis were found in this study it is not surprising that this has not been previously reported where smaller numbers have been examined. This theory of extent of air sac growth determining extent of pneumatisation is also supported by the 3rd cervical vertebrae having a slightly lower incidence than the succeeding members of the series. Similarly the ossa coxarum

being the most caudal bones pneumatised by the abdominal sac have the lowest incidence. The incidence in the femur was nil confirming King (1957) and others and this again is probably an indication of failure of the air sac to grow as far as it does in other species which have pneumatised femora.

The few bones which were constantly pneumatised had, if fully pneumatised, a score of 46. The maximum possible if all variably pneumatised bones were fully involved in one individual was 188. The range was therefore potentially from 46 to 188. In fact the standard deviation of 13.95 was rather smaller than might have been expected.

Correlation between the bones pneumatised by the clavicular sac suggested that individual air sacs might be a principal controlling factor determining the extent of pneumatisation in a particular region of the skeleton. It was found that some adjacent bones in the clavicular sac group, left and right coracoids and right coracoid and right humerus had correlations suggesting that a localised area of air sac might have a particular potential for pneumatisation whereas the more widely separated left humerus and right humerus showed correlations which were significant by one statistical method and beyond the level of significance by the other. These bones are pneumatised by widely separated areas of the clavicular sac, the peripheral parts of the lateral diverticula, and these areas may have less similar pneumatising capacities. The correlation between left humerus and left coracoid was similarly significant by only 1 test although by the other the correlation was rather closer to significance than in the case of left and right humeri. The theory is further substantiated by examination of the vertebral column where groups of adjacent vertebrae show a similar pneumatisation status both in occurrence

and extent. It was very unusual to find an isolated unpneumatised vertebra surrounded by pneumatised, other than at the very periphery of the group as discussed above. The lack of correlation of pneumatisation of the sternum with that of coracoids or humeri is, therefore, difficult to explain. It was noted that the involvement of the length of the sternum in the midsagittal plane was always less than 40% of the total and always involved the most cranial, widest region of the sternal mass. The width of the extent was not investigated and it could be that increasing extent would be more manifest in this direction. Only in 2 cockerels which, as discussed later, were among the most heavily pneumatised birds in the study, had pneumatisation progressed as far laterally as the sternal processes.

The correlation found between cervical and abdominal sac groups is at first rather surprising in view of the general lack of correlation between air sac groups. These 2 sacs are, however, principally involved with the pneumatisation of vertebrae and it may be, therefore, that a degree of uniformity of the process within the vertebral column produces this correlation. The only other correlation found, between the cranial and clavicular groups is rather more difficult to explain.

The examination of total skeletal pneumatisation as performed in this study can be done fairly quickly by the method used. If, however, in future studies of large numbers of birds it was preferred to limit the examination to one region of the skeleton the very close correlation between the cranial region and the total would strongly recommend the skull including mandible, quadrate and pterygoid as the region for selection.

Only 3 cockerels were included in the survey as it was not the main intention to investigate sex differences. However, even in these very

limited numbers, there is a strong indication of greater pneumatisation in these male birds, one having easily the greatest total pneumatisation score and the other 2 being at the upper end of the range for the females in the series. Also as mentioned above only in 2 cockerels were the sternal processes pneumatised, and the basioccipital was fully pneumatised including the condylar part only in cockerels. A greater extent in cockerels would not be surprising, the paranasal sinuses of the mammal tending to have a greater development in the male.

iii. DEVELOPMENT OF PNEUMATISATION IN THE POSTNATAL SKELETON

INTRODUCTION

Information on the development of skeletal pneumatisation in the fowl and indeed in birds in general is extremely sparse. Sappey (1847) described it as a late development, the bones containing marrow throughout their development which lasts from several weeks to 6-8 months after which pneumatisation develops. Coues (1890) described pneumatisation as present in adult life and Bradley (1960) termed it a late development.

For specific bones in the post-cranial skeleton there is virtually no information at all. In the fowl the humerus was said to become pneumatised on the 22nd day (Selenka, quoted by Bradley 1960) and at about the 3rd week (Bremer 1940b). The latter work is one of the few recent scientific reports on development of pneumatisation and has been much quoted subsequently, yet again contains the erroneous statement that the humerus is pneumatised from the cervical sac. Although factors capable of producing variations in time of onset have been suggested there are few actual reports. Pratt and McCance (1960) claimed pneumatisation of

the humerus to occur earlier in fowls which had been undernourished. They also wrongly claimed that the cervical air sac was responsible for humeral pneumatisation, again indicating the confusion still existing on this subject even in recent literature.

The one area to which considerable attention has been paid is the skull where, as has been referred to previously, ornithologists have used development of pneumatisation in the dermatocranium as an ageing index. This has been done since early in the 19th century and continues to be much in vogue. Among recent, detailed reports are those of Nero (1951), House Sparrow, Harrison (1957a, 1960, 1961) Wood Pigeon, doves, Sparrow, Starling and corvidae, Lebermann (1970), Ruby-crowned Kinglet, McNeil and Burton (1972), North American shorebirds, Winkler (1972, Herring Gull, and Biur and Thiaphyal (1972), Indian Weaver bird.

All are based on the degree of transparency of the skull to light. The basis as described by Harrison (1964) is that pneumatised bone is opaque and spongy diploe translucent. Completion is said to take up to 2 years. Most of these reports refer to the observed changes as being due to pneumatisation though Nero (1951) uses the term "ossification". Several, such as Harrison (1960), show spread from frontal to parietal as occurring only after the intervening suture fuses. This view has been advanced by a number of authors as quoted previously in this study.

The skull of the fowl has not been studied in this way, nor have these results been confirmed by direct identification of pneumatisation.

Prenatal pneumatisation of the basis cranii, quadrate and pterygoid has been described by Bremer (1940a). The mandible was considered not to be pneumatised as mentioned previously. De Beer (1937) also described pneumatisation as beginning in the base of the skull before hatching.

The object here was to confirm what pneumatisation was present in the fowl skeleton at the time of hatching and to follow the development in the postnatal period. It was also intended to compare transillumination with section and direct examination as methods for identification of cranial pneumatisation and to investigate the value of radiography as a technique for this purpose where it does not appear to have been utilised. As in previous parts of this study it was not the main intention to study degree of variation beyond that occurring in a similar group of birds due to individual genetic differences, but in this case it was thought that suitable birds were available to permit limited investigation of difference between the sexes and 2 breeds.

MATERIALS AND METHODS

Skull

White Leghorn birds were sampled in pairs, 1 male and 1 female at hatching and at 7 day intervals to 119 days and the last male bird at 126 days. The skulls were sectioned midsagittally and examined under the operating microscope as in the adult survey for evidence of pneumatisation. In the younger members of the series the joints between bones could be identified and the structure of the parietal and frontal whether in the form of a thin single plate, or thickened, or with the development of diploe was noted. A series of drawings was prepared showing these features and the localisation of any pneumatisation present.

After removal of skin, eyes and cranial contents the 2 halves of the skull were apposed and transilluminated. The outline of the dermatocranium and areas of translucence were drawn alongside the previous series

of drawings.

In the birds killed at hatching portions of the individual cranial bones and mandible, quadrate and pterygoid were sectioned and examined histologically to determine the extent of the pneumatisation at this time.

The silver nitrate radiographs of the Golden Comets used in examination of skull centres and neurocranial fusions were examined for evidence of pneumatisation. While this could sometimes be identified quite clearly in some regions it was felt that it did not provide a sufficiently reliable guide for accurate study of the problem and was not, therefore, pursued further.

Postcranial skeleton

Golden Comet pullets were sampled in pairs at hatching and at 7 day intervals till 112 days then at 14 day intervals till 182 days. All bones previously found to be pneumatised in the adult survey, and the sternal ribs were studied similarly by section and direct examination.

In the White Leghorn birds of which the skulls were used the left wing was radiographed laterally keeping the wing as close to the plate as possible. Subsequently the left humerus and coracoid of these birds were sectioned and examined.

RESULTS

a. Skull

The results obtained from examination of the sectioned skulls are shown in fig. 118a-d. At hatching pneumatisation was confined on the midsagittal section to the supraoccipital and parasphenoid/basisphenoid. Histological examination confirmed the occurrence of pneumatisation in

these bones and also identified it as having developed in the squamosal, exoccipital, quadrate, pterygoid and mandible. The basioccipital was first found to be pneumatised at 7 days postnatal and then variably up to 28 days postnatal after which it was invariably pneumatised.

The parietal was seen at hatching as partially a single thin plate of bone and partially as containing diploe. At 42 days and thereafter the entire bone contained diploe. Pneumatisation was first observed within diploe at 28 days postnatal (birds F28, M28) and in all birds aged 42 days and over. It was first seen to be complete at 42 days postnatal (bird M42). Pneumatisation sometimes appeared to develop first in the anterior part of the bone and not to spread rostrally from the supraoccipital, the pneumatisation of which stopped abruptly at the suture between these bones. This joint and the frontal-parietal were observed in all birds up to 77 days and had fused in all birds aged 105 days and over.

In the frontal the structure was of a single thin plate of bone at hatching which gradually thickened up to 35 days postnatal when diploe started to appear, with red marrow visible in the spaces in the cancellous bone. The first signs of pneumatisation were seen at 42 days (bird F42) and intermittently but in gradually increasing amounts thereafter, until the adult pattern was attained although in a few cases even in older birds in the series (F77, M119) pneumatisation was entirely absent.

The orbitosphenoid and mesethmoid were found only to be pneumatised in the oldest birds in the series.

By transillumination it was found that most younger birds in the series showed 3 fenestrae, a single in the parietal and a larger pair in the frontals as shown in fig. 118a. After 42 days the parietal fenestra

was always absent. There was also a reduction in size of the frontal fenestrae and from about 49 days onwards only the various stages found in the adult survey shown in fig. 105 and a few slight variants were seen. Several belonging to stage 6 were found having no fenestrae present.

b. Vertebral column

Cervical region

The pattern of development of pneumatisation is shown in fig. 119.

All birds examined had 16 cervical vertebrae.

No pneumatisation was found until 35 days postnatal when it occurred in 1 bird in cervicals 8-13 and cervicals 3, 4, 6-13 and 16 in the other. Thereafter cervicals 5-12 were constantly pneumatised. The first signs appeared in cervicals 14, 15 at 42-49 days and the last 4 cervical vertebrae were more or less constant in pneumatisation from 56 days postnatal onwards. Cervicals 3 and 4 were constant from 63 days onwards.

The usual adult pattern was achieved fairly early, in that in most cases from 63 days onwards cervicals 3-16 were all fully pneumatised.

Partial pneumatisation was virtually only seen up to 49 days postnatal.

Thoracic region

The pattern of development of pneumatisation is shown in fig. 120.

All birds had 5 vertebrae.

No pneumatisation was seen until 63 days postnatal when it occurred in thoracic 1 in one bird. The next occurrence was in thoracics 1, 4 and 5 at 77 days after which it was almost constant in thoracics 4 and 5 but continued to be variable in thoracic 1 until the upper end of the

series. No evidence of pneumatisation of thoracics 2 and 3 was found except in 1 bird at 154 days postnatal.

In most cases when pneumatisation occurred it involved the vertebra fully.

Lumbosacral region

The pattern of development of pneumatisation is shown in fig. 121.

All birds had 15 lumbosacral vertebrae.

No pneumatisation was found till 77 days postnatal when it commenced in the cranial members of the series (lumbosacrals 1-5) being fairly constant thereafter. In lumbosacrals 6-8 it commenced at 126 days and caudal to that was only found at 182 days. In numbers 11 and 12 it was not identified at all.

c. Ribs and sternum

The pattern of development of pneumatisation is shown in fig. 122.

In the vertebral ribs pneumatisation was seen in the first at 91 days postnatal and frequently thereafter but in the second was only seen on one occasion at 154 days postnatal. It was not found in any of the other vertebral ribs or in the sternal ribs.

In the sternum pneumatisation was detected in birds aged 140 days and upwards.

d. Os coxae

No pneumatisation was found.

e. Coracoid

In the Golden Comet birds no pneumatisation at all was found. The

pattern of development in the White Leghorns is shown in fig. 123.

Pneumatisation was first found at 63 days postnatal (F63, M63) and was found in all birds aged 84 days and over. The extent increased though subject to considerable variation until 91 days after which it was found to involve 60-80% of the bone length in all birds examined thereafter.

The difference in rate of development in the male and female birds was examined by a paired t test and was found to be not significant ($P \geq 0.10$).

f. Humerus

The pattern of development of pneumatisation assessed by section in Golden Comets and by section and by radiography in White Leghorns is shown in fig. 124.

In the Golden Comets pneumatisation was first found at 35 days postnatal and in all subsequent cases except 3. The extent gradually increased though subject to considerable variation and in all birds after 112 days it was 80% or more when it occurred.

In the White Leghorns when examined by section pneumatisation was first seen in a 28 day old bird (F28) and in all examined thereafter. The extent increased rapidly and all birds except 1 aged 84 days or over it exceeded 80%. Up to 112 days the extent in females appeared to exceed that in the corresponding male. After this both sexes appeared to achieve a similar final maximum. The difference in rate of development in the pairs of males and females up to and including 119 days postnatal was investigated by a paired t test. This difference was found to be highly significant ($P < 0.01$).

The rate of development in the Golden Comet females appeared rather slower than in the White Leghorn females. This was also compared using a paired t test and was found to be highly significant ($P < 0.001$).

Radiographically, pneumatisation was identified as an area of reduced density as shown in fig. 125. When the results for the White Leghorns derived by the 2 methods were compared it was found that pneumatisation was similarly identified as present or absent by both techniques. The final extent was usually slightly greater when assessed radiographically and in the developing period the difference appeared rather greater.

DISCUSSION

Prenatal development of pneumatisation in the parasphenoid/basisphenoid, supraoccipital, pterygoid and quadrate was confirmed. The involvement of the articular region of the mandible agreed with the results in the adult survey in this study though disagreeing with Bremer (1940a).

The fusion times of the joints identifiable on the section surface of the neurocranium generally agreed with those found in part 2 of the study, although in this case it was not possible to follow the joint line throughout its length to assess accurately the onset of fusion.

The extent and variability of pneumatisation achieved in the frontal broadly agreed with the results of the adult survey. No obvious differences in development were observed between male and female birds in the frontal nor indeed elsewhere in the skull.

The orbitosphenoid became pneumatised at ages when it had previously been found to have fused to adjacent elements. Similarly the mesethmoid was found to become pneumatised late after fusion would have been

completed. This supports the view that fusion is a necessary prerequisite for spread of pneumatisation across intervening joints which in these cases are synchondroses. On the other hand the parietal was first seen to be pneumatised at 28 days postnatal, well before the fusion of its sutures occurs. Unless pneumatised directly from the tympanic cavity, which seems in this location most unlikely, there must be direct spread from adjacent bones. The frontal is not yet involved and there is no evidence of spread from the supraoccipital as the first signs were often seen in the central part of the parietal and pneumatisation of the supraoccipital was found to cease abruptly at the parietal-supraoccipital joint (fig. 118a, M28, F28). The most likely route would seem to be from the squamosal which forms part of the boundary of the tympanic cavity and is pneumatised at hatching. The suture between the squamosal and the supraoccipital is very narrow as noted previously and these bones are united by fibrous connective tissue, since they both ossify in membrane. If the frontal is pneumatised from the tympanic cavity as suggested by Bremer (1940a) the method of spread must be similar, as pneumatisation occurs well before suture fusion. It may be, therefore, that the pneumatisation process can penetrate through true sutures, being fibrous connective tissue, but not through cartilage. Bremer (1940a) described the onset of pneumatisation in intramembraneously formed bones as being early in the membrane stage and keeping pace with the bone development. This may be true for the skull elements pneumatised early in the prenatal period such as parasphenoid and squamosal but it is certainly not true for the parietal and the frontal. In skull bones preformed in cartilage he described invasion as occurring when the calcified cartilage was removed rather than actual penetration of cartilage. Cortical bone

developed around the air sac process, resulting in the formation of a pneumatic foramen. Bremer (1940b) studied the humerus, as a postnatally pneumatised bone, and described changes reminiscent of osteitis fibrosa cystica in man. Destruction of bone tissue and its replacement by mesenchymal connective tissue preceded the ingrowth of the air sac diverticulum, again suggesting that fibrous connective tissue is the medium through which air sacs will spread. Bremer suggested that oestrogens, derived from the retracted yolk sac before hatching, acted directly or indirectly on the parathyroid glands. The oestrogen theory would go a long way to explaining the precocious development of pneumatisation in the humerus of female birds compared with male. Bremer's evidence is based on the injection of white rats with theelin, an oestrogen occurring in the yolk sac. This produced bone changes similar to those found in the humerus of chicks at the onset of pneumatisation. However, there are several major weaknesses in the theory. Firstly an attempt by Bremer (1940b) to prevent the onset of pneumatisation in chicks by treatment with androgens was unsuccessful and pneumatisation proceeded normally. Secondly, the hormonal control of medullary bone development in birds reviewed by Taylor et al (1971) contradicts Bremer's theory. It has been shown that oestrogens and androgens act synergistically to produce osteogenesis prior to ovulation. Although there is some dispute over the mechanism controlling the bone resorption occurring after ovulation there seems to be no evidence to suggest that oestrogens are responsible. Finally, although Bremer's (1940b) work was performed on the humerus, he clearly believed that the mechanism was similar for the entire pneumatised skeleton. However, this study has shown that the humerus is, in fact, among the earliest bones postnatally pneumatised and some such as the sternum and

caudal lumbosacral vertebrae do not become involved until near the end of the growth period. It is very hard, therefore, to believe oestrogens of yolk sac origin would have any function in these instances. The controlling mechanism of the process is clearly in need of much further attention.

Although the first evidence of pneumatisation in the frontal was found by section at 42 days postnatal the roof of the cranium in fact was never completely transparent to light in any bird from hatching upwards, since there were always paired fenestrae with intervening opacity in the midline. This corresponded with a ridge which progressively thickened to form an internal sagittal crest. In the parietal the single fenestra corresponded to the area where the bone was in the form of a single thin plate. After 35 days postnatal the frontal fenestrae rapidly decreased in size and diploe developed, soon followed by traces of pneumatisation but this progressed much more slowly than the reduction in size of the fenestrae. In fig. 118b it can be seen that in bird M56 complete opacity with total lack of fenestrae had been achieved but only a small area of pneumatisation in the most rostral part of the frontal was found. Bird F77 in fig. 118c only showed small paired frontal fenestrae yet no pneumatisation was seen by section at all. It is concluded therefore that opacity of the skull is due either to simple thickening of the bone while still in the form of a single plate or to formation of double plate structure with intervening diploe in which pneumatisation may be present or absent. The method cannot be regarded as sound for accurate identification of pneumatisation which only occurs after the formation of diploe if it occurs at all. This is not to say that the method is not of use in ornithology for assessing age but the index being used is one of <u>skull</u>

maturation rather than pneumatisation.

The most cranial and caudal members of the cervical vertebrae were found to become constantly pneumatised slightly later than the remainder probably due to the cervical air sac not having reached these peripheral vertebrae at the time its diverticula start to penetrate. The first thoracic is also pneumatised from this sac and this commences slightly later than the last cervical. The 2nd and 3rd thoracics are also pneumatised by this sac although it only extends as far caudally as the 1st thoracic (King 1975). These commenced their pneumatisation only towards the upper end of the series after the time of fusion of the joints of the notarium and points to a spread of pneumatisation caudally from the 1st thoracic. This is supported by thoracic 3 having the lowest incidence in the adult survey followed by thoracic 2 which is lower than thoracic 1. It was also the case that thoracics 2 and 3 were never pneumatised in the absence of thoracic 1. This route also explains the fact that in partial pneumatisation of the 3rd thoracic this occurs in its cranial part as shown by King and Kelly (1956). The 4th and 5th thoracics become pneumatised from the abdominal sac (King 1975) and this was found to occur relatively early and with virtually no subsequent variation. At the same time pneumatisation of lumbosacrals 1-5 commenced simultaneously but from here only spread caudally much later and to a variable extent. This is thought again to be occurring subsequent to fusion of these vertebrae. This failure to spread between vertebrae prior to synostosis is further indication of the inability of the process to invade cartilage.

After the time of its onset pneumatisation of the coracoid in the White Leghorns seemed of much more constant occurrence than in the adult Golden Comet series. Although the extent was still subject to some

variation it exceeded 60% in the older birds in the series whereas it never exceeded 40% in the adult Golden Comets. It was remarkable that pneumatisation was not seen at all in the coracoids of the growing Golden Comet series. It could be that this had yet to commence at the upper limit of the series but as this would be so very much later than the onset in the White Leghorns it does seem unlikely. Only 7 out of 48 adult hens had shown pneumatisation in the left coracoid and in the limited numbers used in the growing series it may be that all birds in the critical period were of the unpneumatised category.

The humerus also was constantly pneumatised in the White Leghorns after it was first detected whereas several Golden Comets remained unpneumatised as in the adult survey. The White Leghorn females also showed more rapidly developing pneumatisation than Golden Comet females which was surprising as the 2 groups are both fairly similar laying types. It was more rapid in development in the White Leghorn females than males.

The results obtained by radiography of the humerus were in fairly close agreement with those given by section. A slightly greater maximum extent was recorded by radiography due to the total length measurement being limited to the ossified shaft whereas by section the articular cartilages were included. The greater disparity during the growing period might be explained by a loss in density of the cancellous bone as it becomes affected by the changes described by Bremer (1940b) in advance of actual invasion by air sac. Radiography of the humerus would appear to offer a satisfactory in vivo method for detection of pneumatisation permitting sequential examination of the changes in the same birds.

Further studies could usefully be undertaken to investigate the effects of physiological and pathological factors on the basic pattern

established in this study. The histological details of the development of skull pneumatisation from tympanic cavity could profitably be reinvestigated with particular reference to invasion of individual bones, especially the mandible, and the manner of spread between bones.

CONCLUSIONS

The gross and microscopic appearance of pneumatised bone in various situations has been shown.

In the case of the skull prior injection via the auditory tubes was found to offer no advantage over direct examination of the sectioned surface. Histological examination confirmed the results gained by gross study but additionally identified pneumatisation in one prefrontal. Transillumination of the macerated skull was not found to reveal pneumatisation accurately. Radiography was found to be a satisfactory alternative for identification of pneumatisation in the humerus but not sufficiently accurate for detailed study of the skull.

Pneumatisation was constantly found to involve all bones of the neurocranium including the interorbital septum and the quadrate. The prefrontal and pterygoid were variably involved as was the articular region of the mandible, contrary to Bremer (1940a).

The occurrence of pneumatisation in the adult postcranial skeleton was generally similar to that described by Campana (1875), King and Kelly (1956) and King (1957). In the larger number of birds used in this study only cervical vertebrae 5-9 were constantly pneumatised. Consequently there was a large number of variably pneumatised bones, including the humerus, contrary to all previous reports, and the coracoid in which

the incidence was very low. The axis was occasionally pneumatised but the sternal ribs were not.

In individuals, correlation in extent was found in most of the bones pneumatised by the clavicular air sac. Some correlation was revealed between air sac groups of bones. The cranial region was most closely correlated with total pneumatisation.

The sequence of development of pneumatisation has been followed from hatching when it occurred only in the skull in parasphenoid/basisphenoid, supraoccipital, exoccipital, squamosal, quadrate, pterygoid and mandible to 182 days by which time it had developed in all bones where it had been found in the adult survey, with the exception of the axis, LS 11-12, vertebral rib 3, the coracoid and os coxae where it was thought that low incidence of occurrence had prevented identification.

There was indication that the developing pneumatisation process did not invade cartilage but might penetrate fibrous connective tissue in sutures.

The limited data available pointed strongly to more extensive adult pneumatisation in males than in females although there was significantly more rapid development in the humerus in females than in males.

Development of pneumatisation in the humerus was significantly more rapid in White Leghorn females than in Golden Comet females.

APPENDIX 1

Method of alizarin red S staining

Technique based on method of Dawson (1926).

1. Skin and eviscerate specimen.
2. Fix in 95% alcohol for 24 hours.
3. Digest in 2% potassium hydroxide for 1-3 days.
4. Stain in 1:10,000 alizarin red S in 2% potassium hydroxide for 6-8 hours.
5. Return to 2% potassium hydroxide for 24 hours.
6. Clear in progressively stronger solutions of glycerin.
7. Store in 100% glycerin.

APPENDIX II

Method of silver nitrate impregnation

Technique as described by Hodges (1953).

1. Skin and eviscerate specimen.
2. Fix in 95% alcohol for 48 hours.
3. Immerse in 0.5% solution of silver nitrate for 2-3 days or longer if necessary.

APPENDIX III

Technique of radiography

Apparatus	Dean D44.
Kilovoltage	55-70, dependent on size of specimen.
maS	100-130.
Focal-film distance	85 cm.
Film	Kodirex rapid non-screen.

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A STUDY OF POSTNATAL SKELETAL DEVELOPMENT IN THE DOMESTIC FOWL (*Gallus gallus domesticus*)

TWO VOLUMES

by

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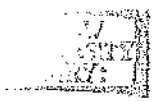
VOLUME II

A thesis submitted for the Degree of Doctor of Philosophy in the Faculty of Veterinary Medicine of the University of Glasgow.

Research conducted in
The Department of Veterinary Anatomy,
University of Glasgow.

July 1977



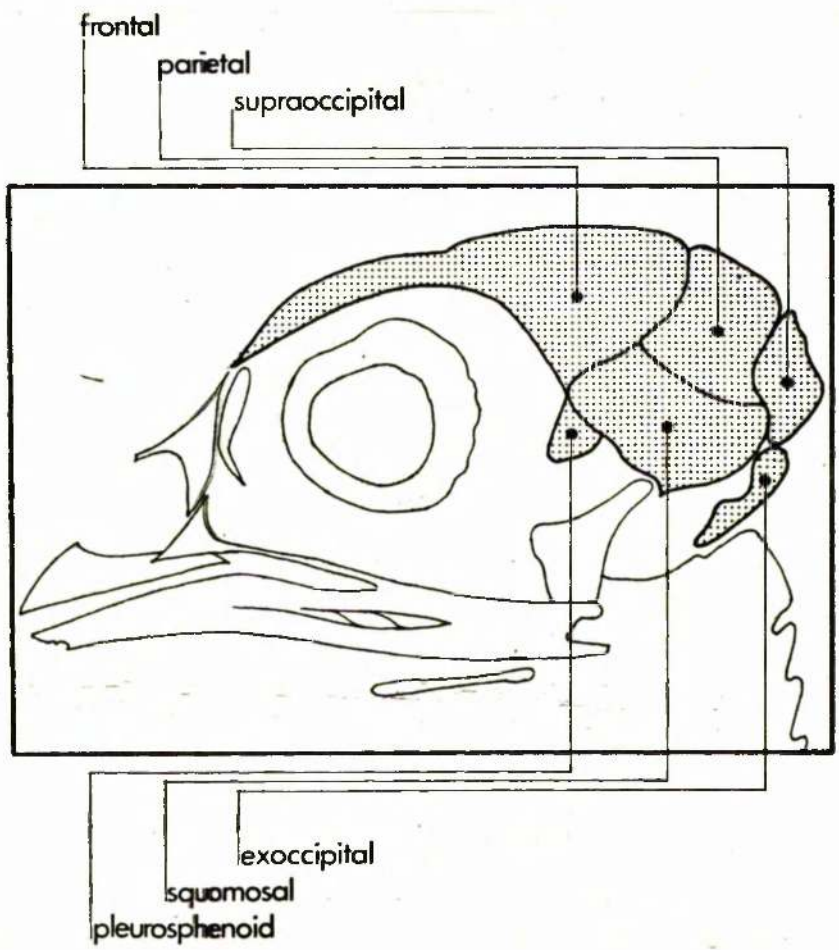


Fig. 1 Skull 0 days
Left lateral view
Alizarin

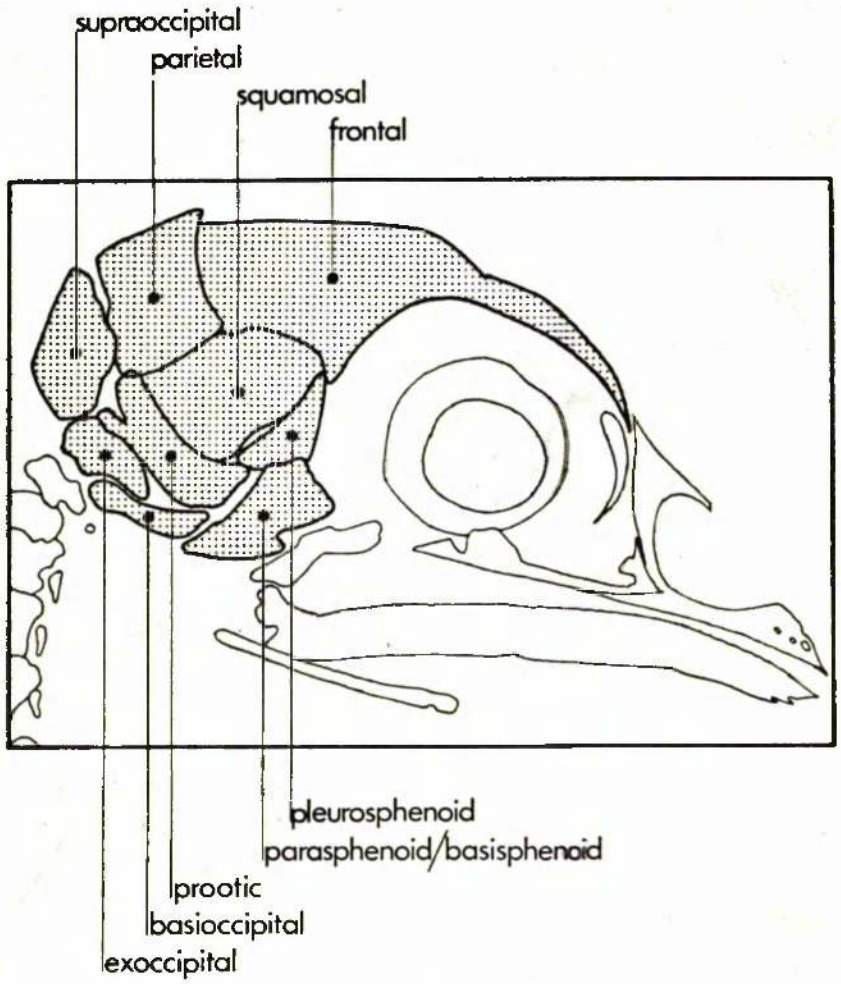
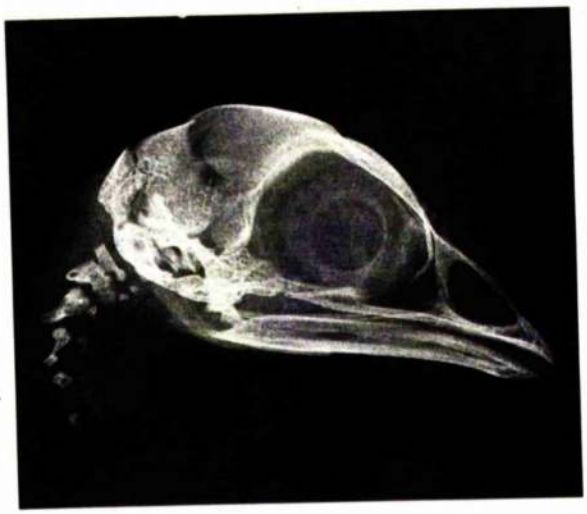


Fig. 2 Skull 0 days
Medial view of midsagittal section
Alizarin



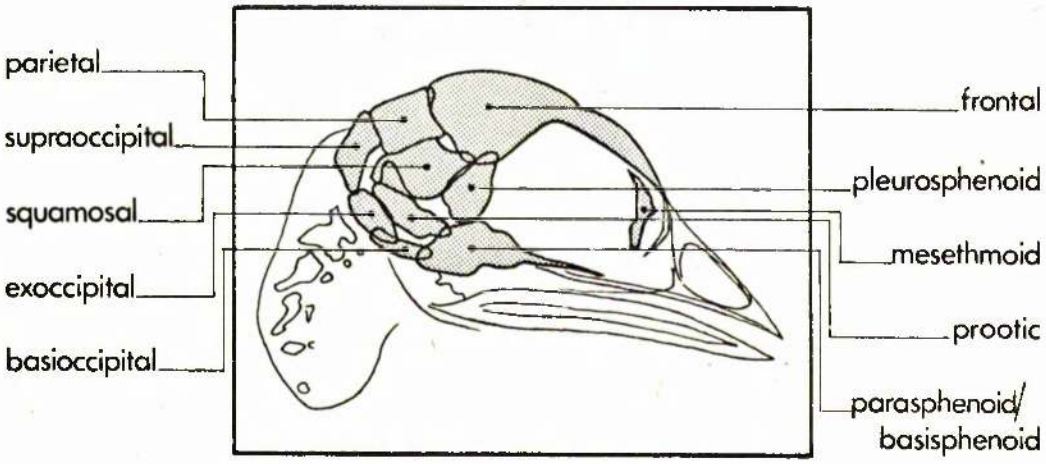


Fig. 3 Skull 0 days

Lateral view of midsagittally sectioned skull

Silver nitrate/radiography

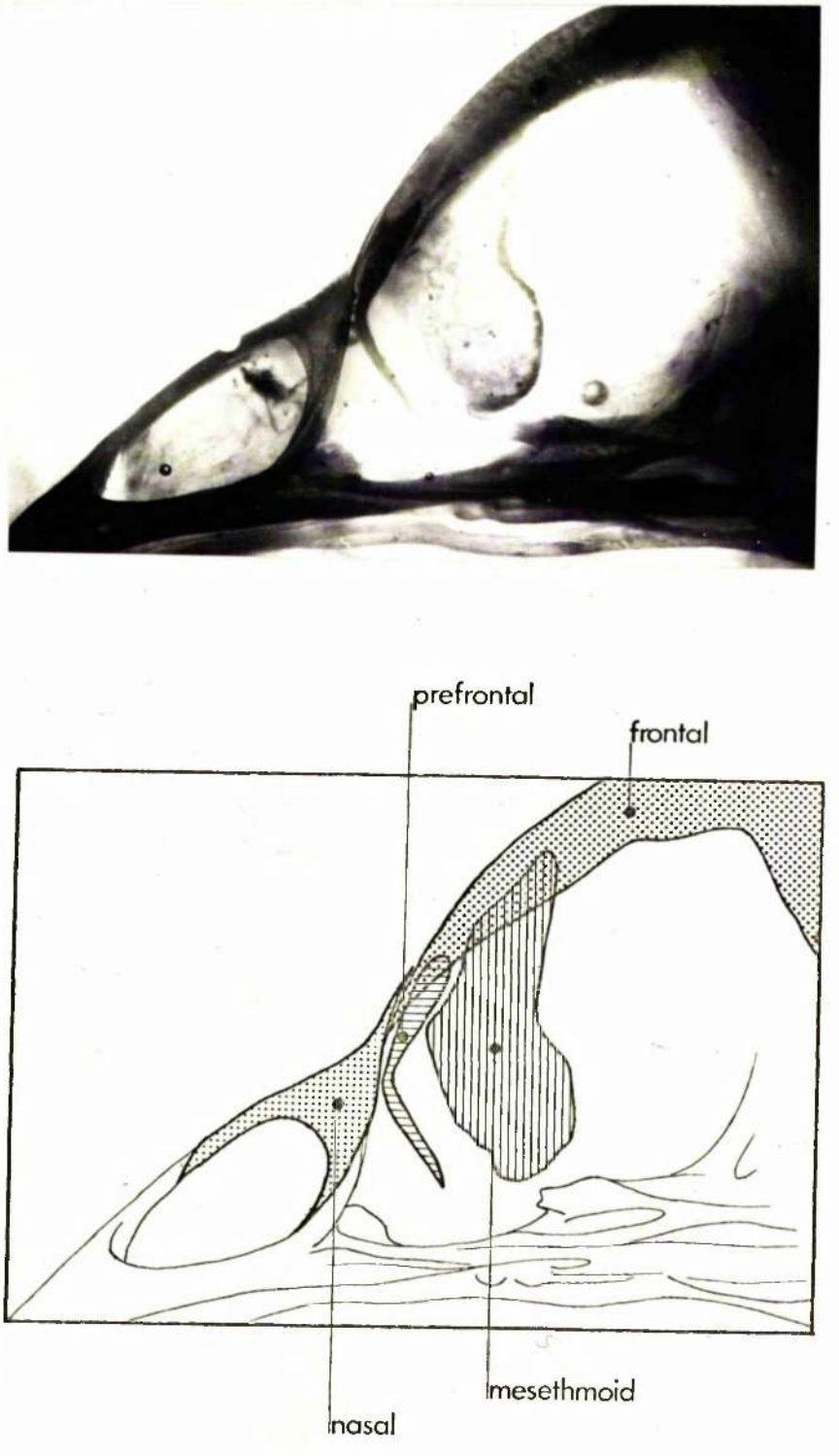


Fig. 4 Skull 0 days
Lateral view showing relations of mesethmoid
Alizarin

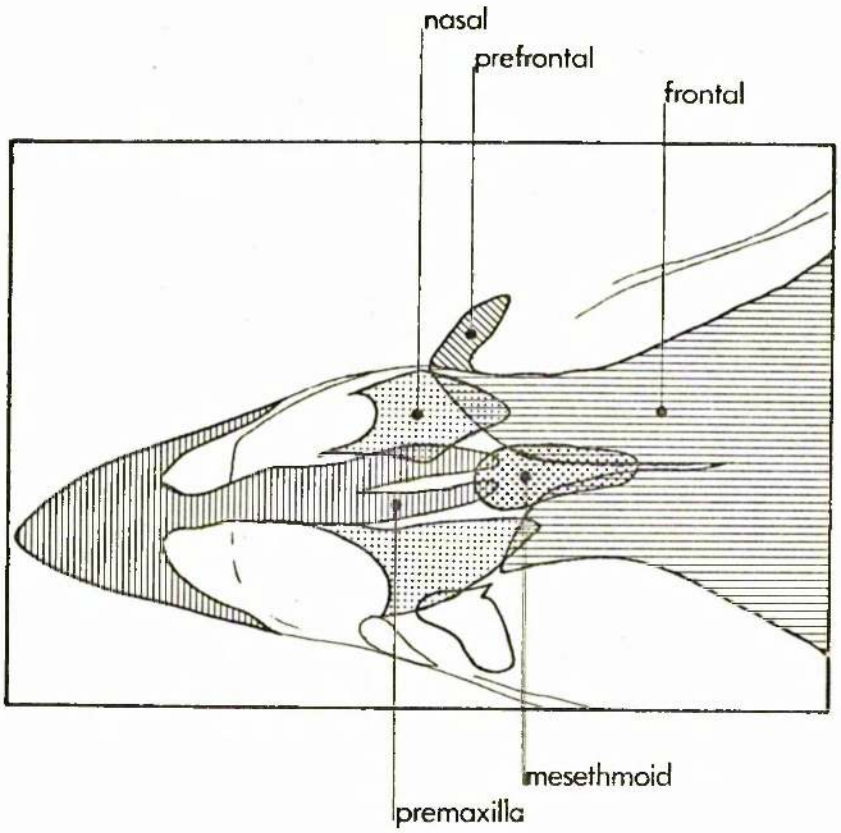
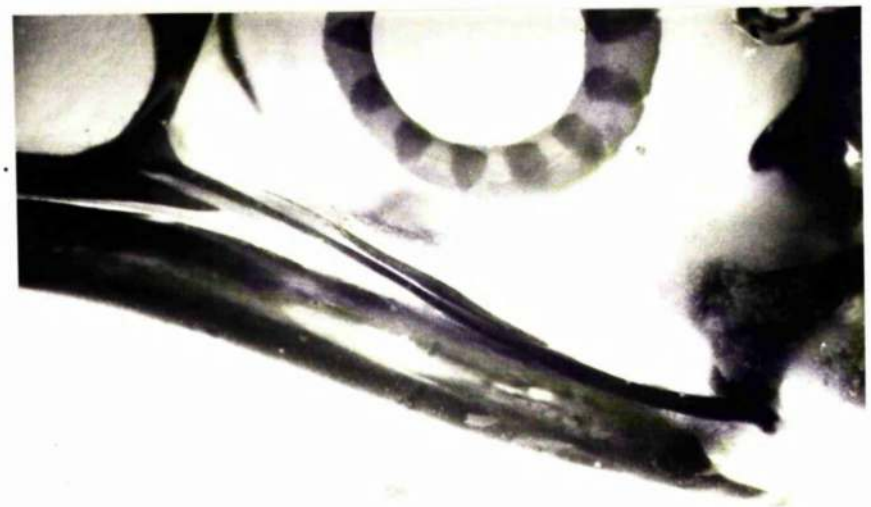


Fig. 5 Skull 0 days

Dorsal view of mesethmoid and its related bones

Alizarin



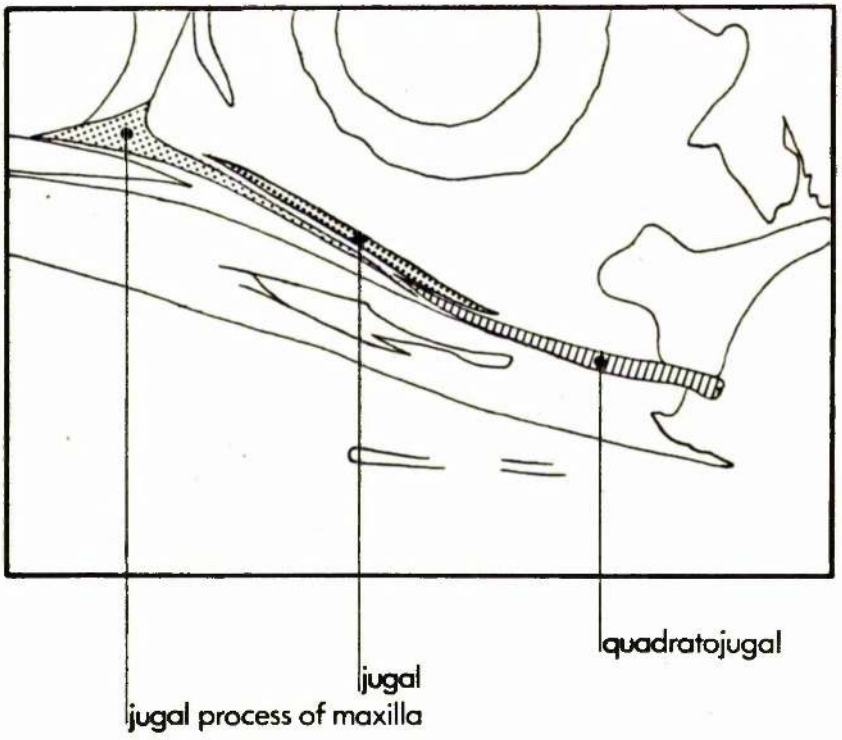


Fig. 6 Skull 0 days
Lateral view of jugal arch
Alizarin

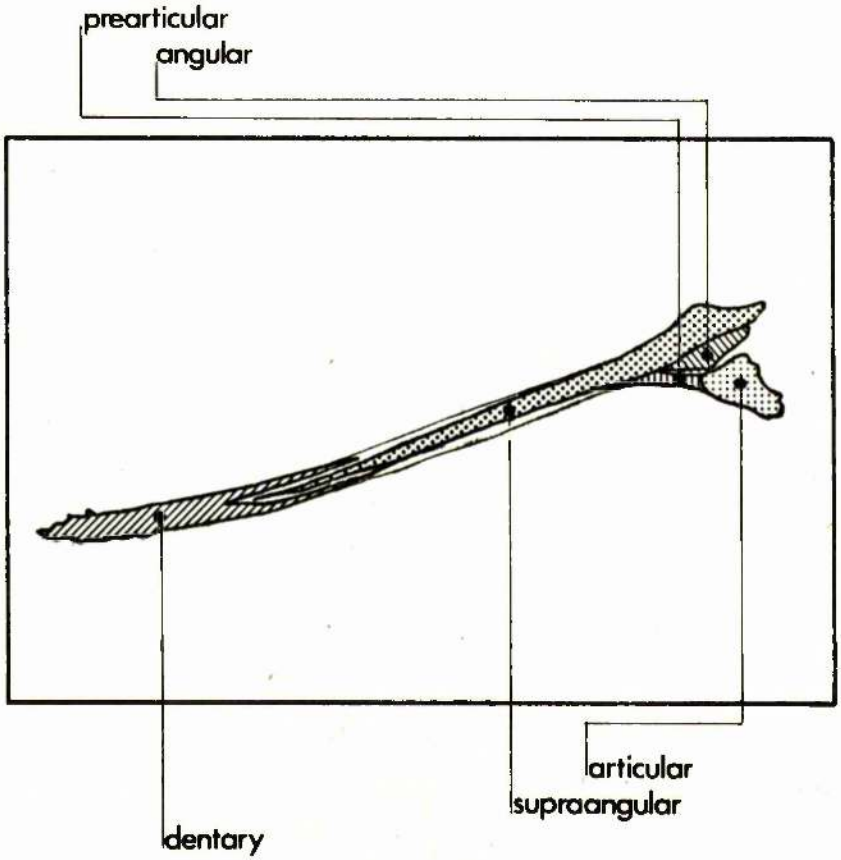


Fig. 7 Right mandible 0 days
Dorsal view
Alizarin

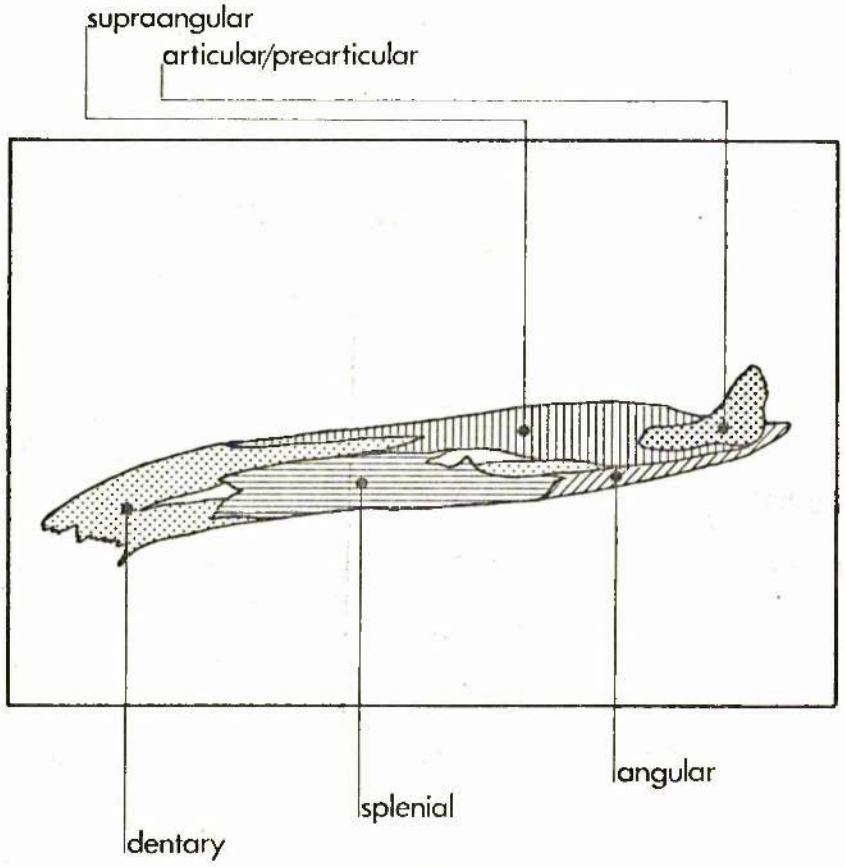


Fig. 8 Right mandible 0 days
Medial view
Alizarin

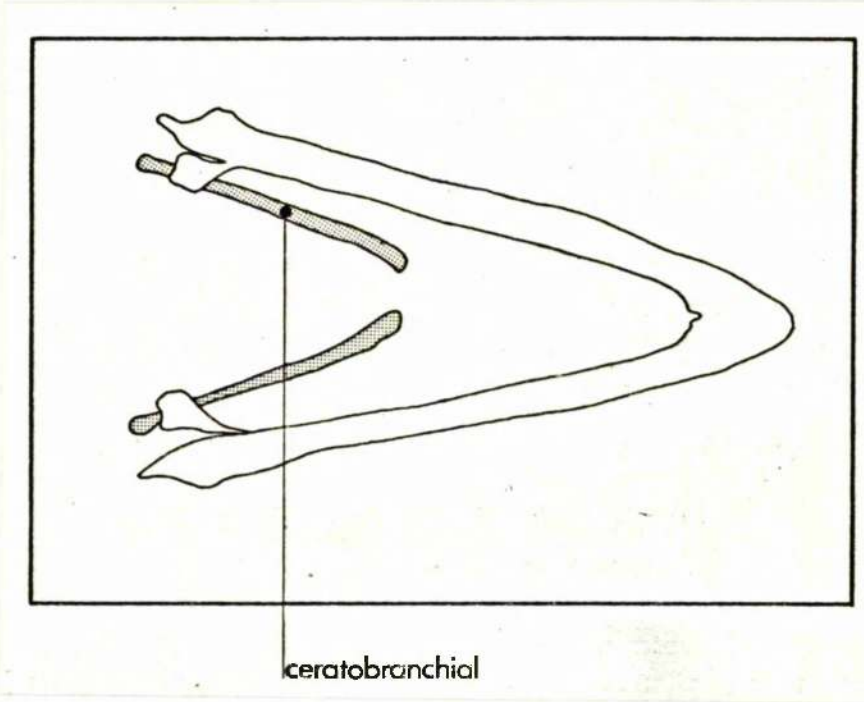


Fig. 9 Mandible and hyoid 0 days
Dorsal view
Alizarin

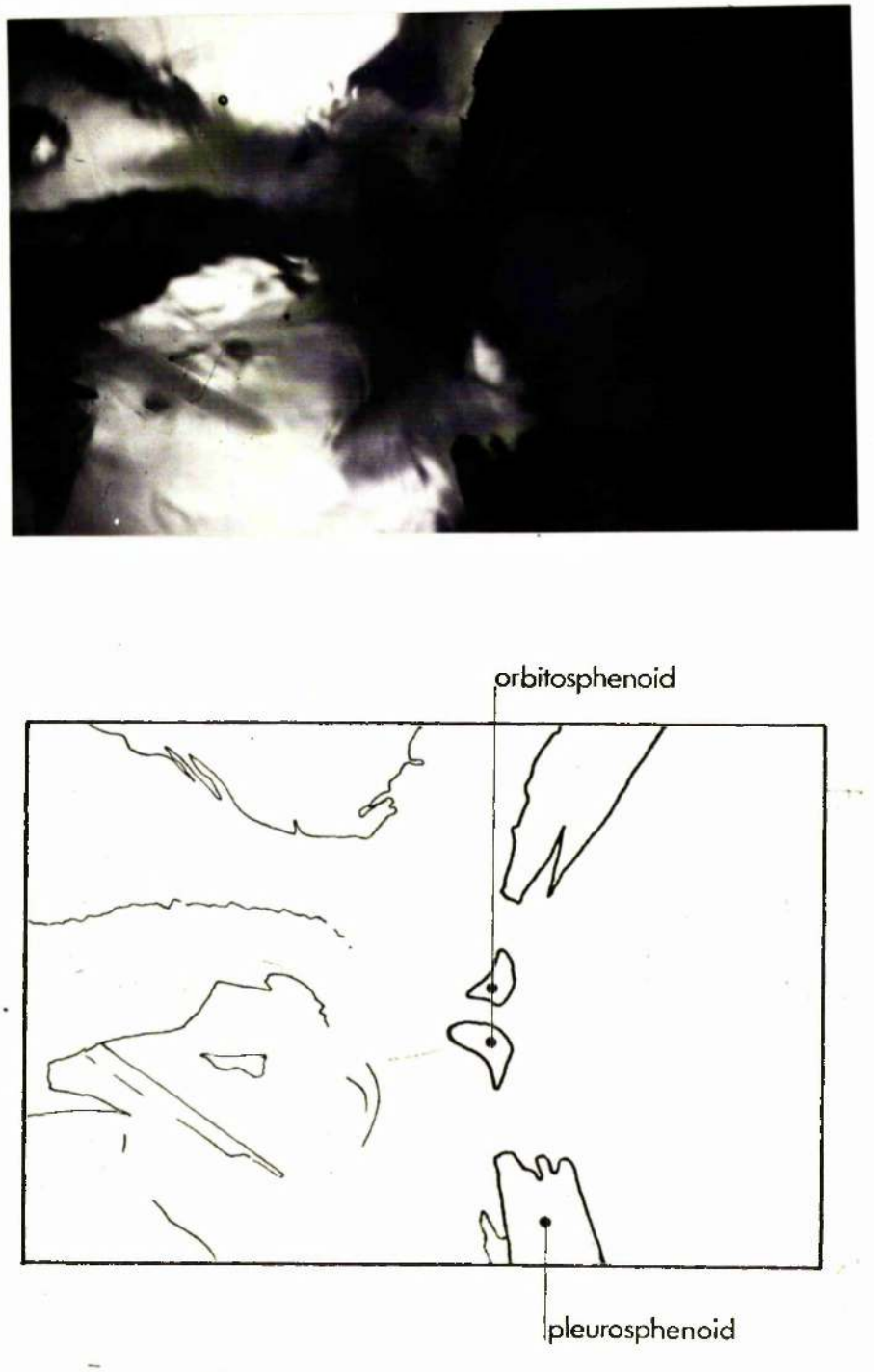
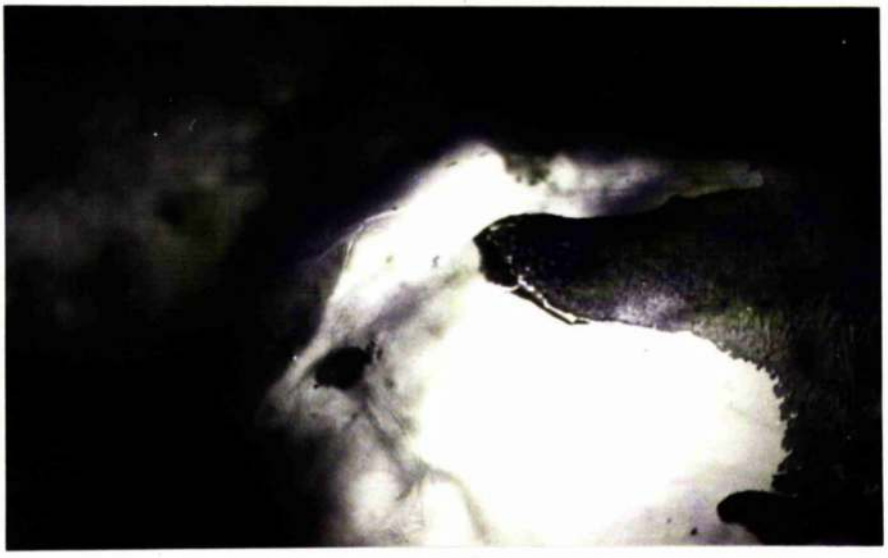


Fig. 10 Base of skull 84 days
Dorsoventral view
Alizarin



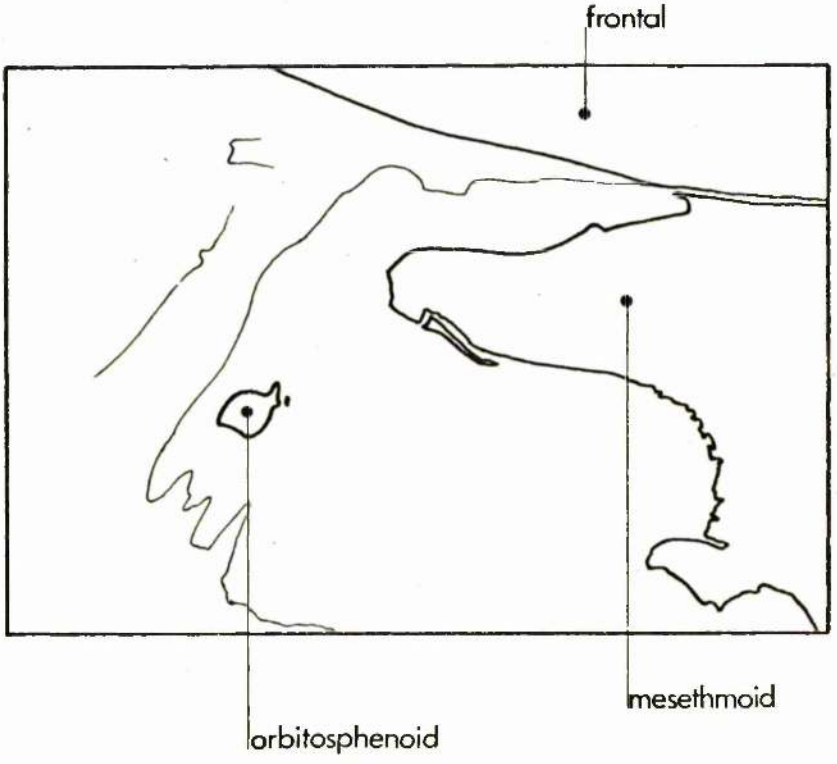
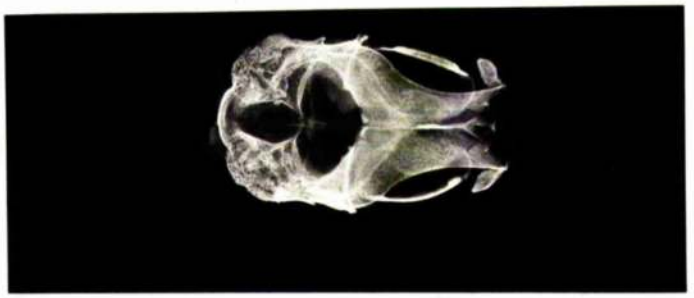


Fig. 11 Skull 84 days
Right lateral view of interorbital septum
Alizarin



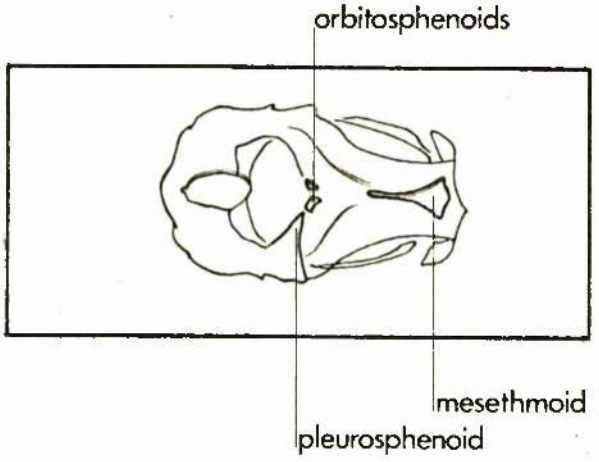
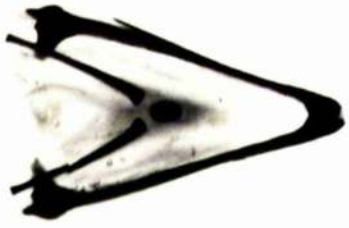


Fig. 12 Skull 105 days

Dorsoventral view of roof of cranium removed by horizontal section through level of optic foramina

Silver nitrate/radiography



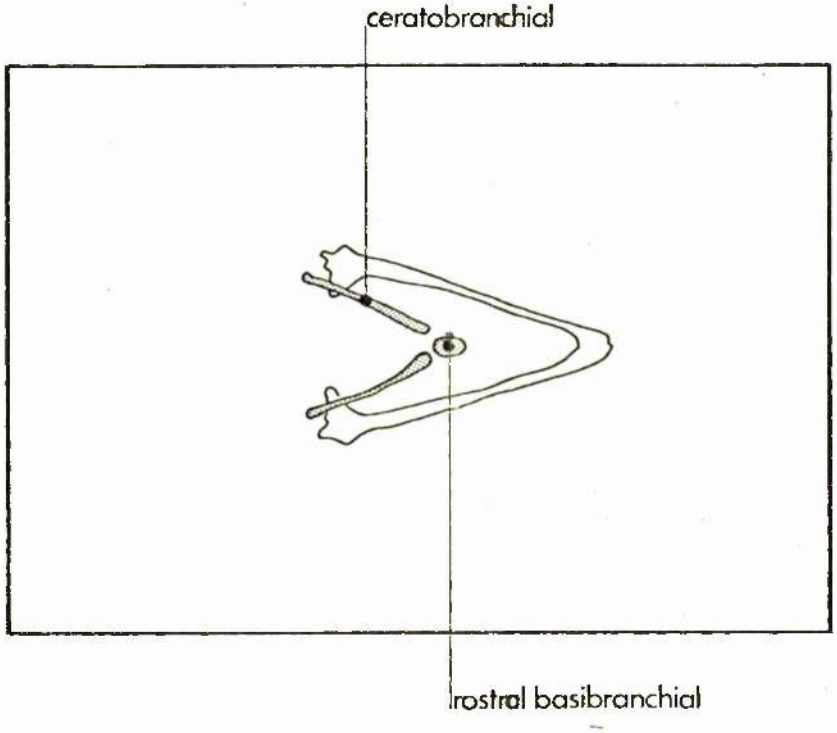


Fig. 13 Mandible and hyoid 56 days
Ventral view
Alizarin

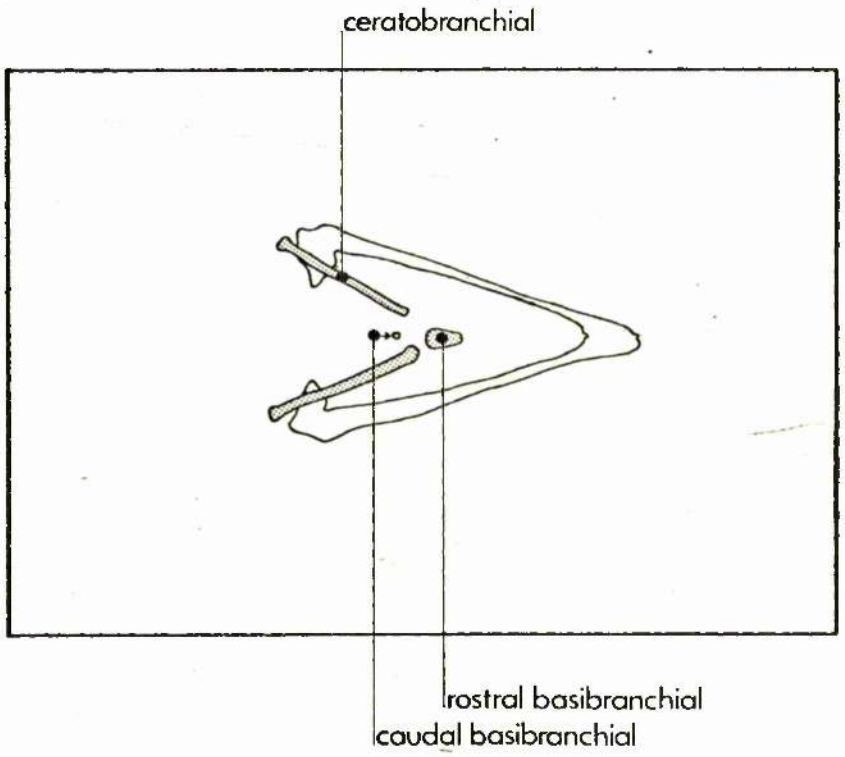
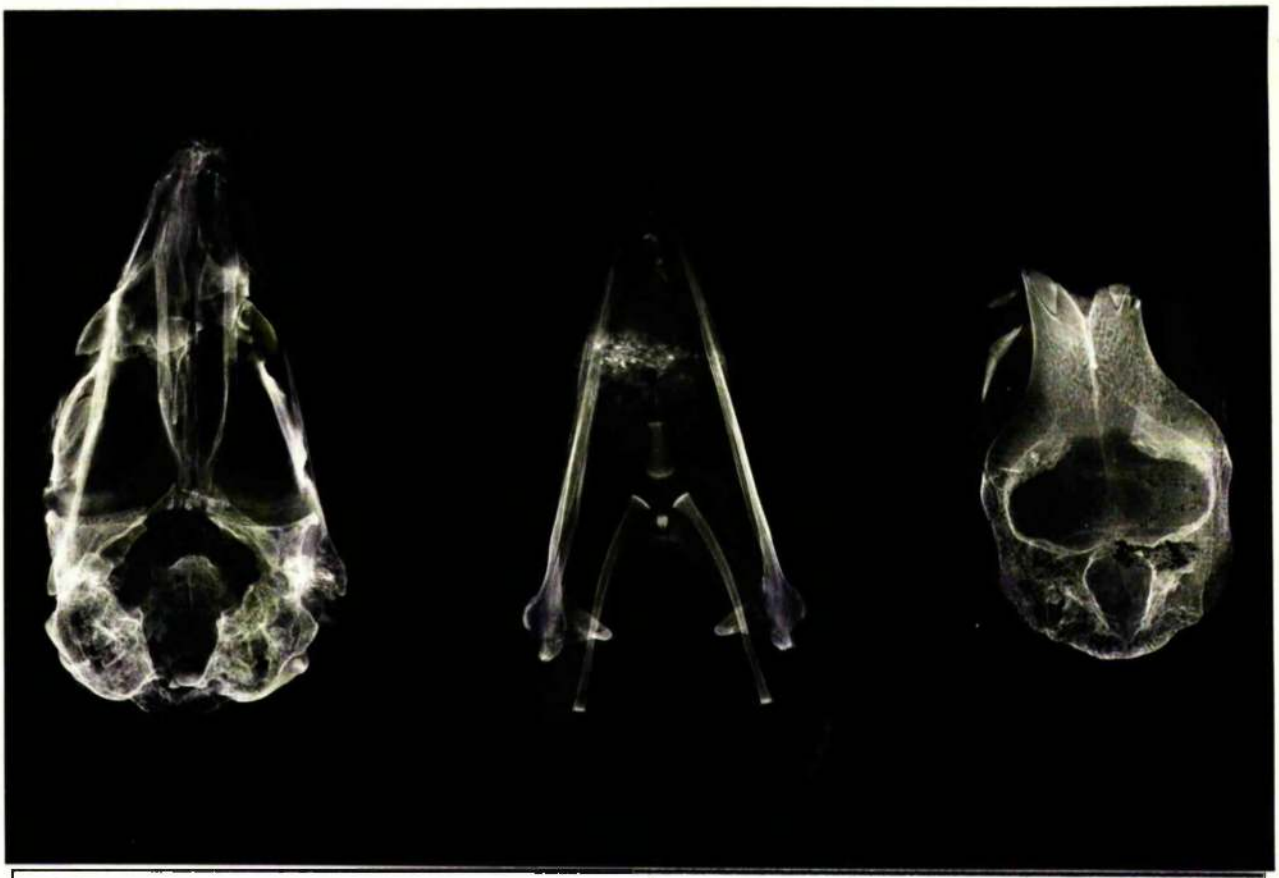


Fig. 14 Mandible and hyoid 70 days
Ventral view
Alizarin



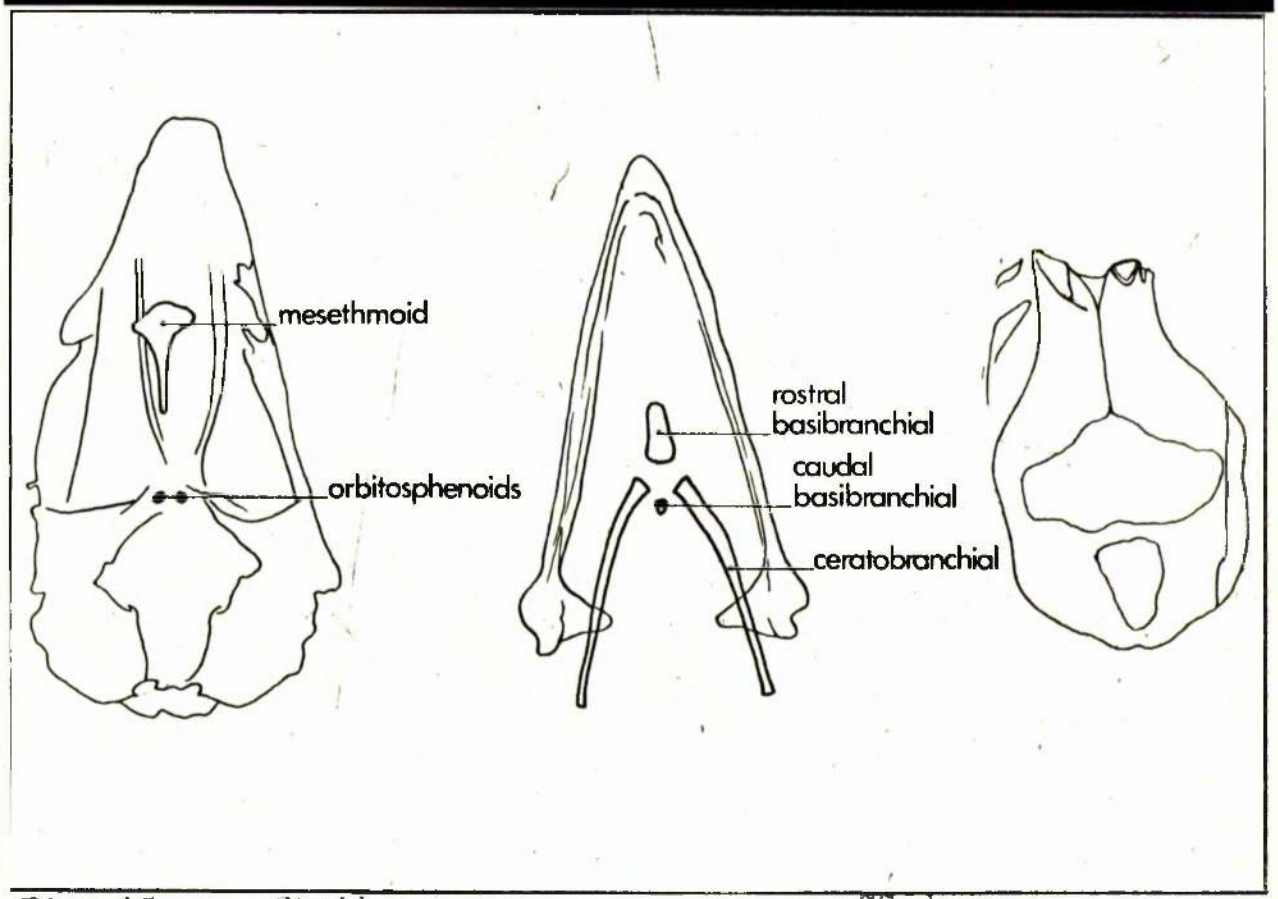
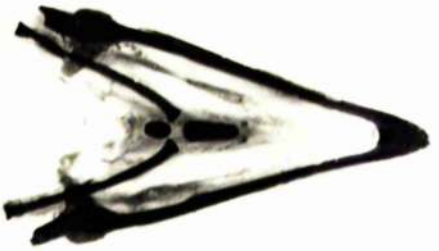


Fig. 15 Skull 77 days

Dorsoventral views:

Left floor of cranium and facial bones
Middle mandible and hyoid
Right roof of cranium

Silver nitrate/radiography



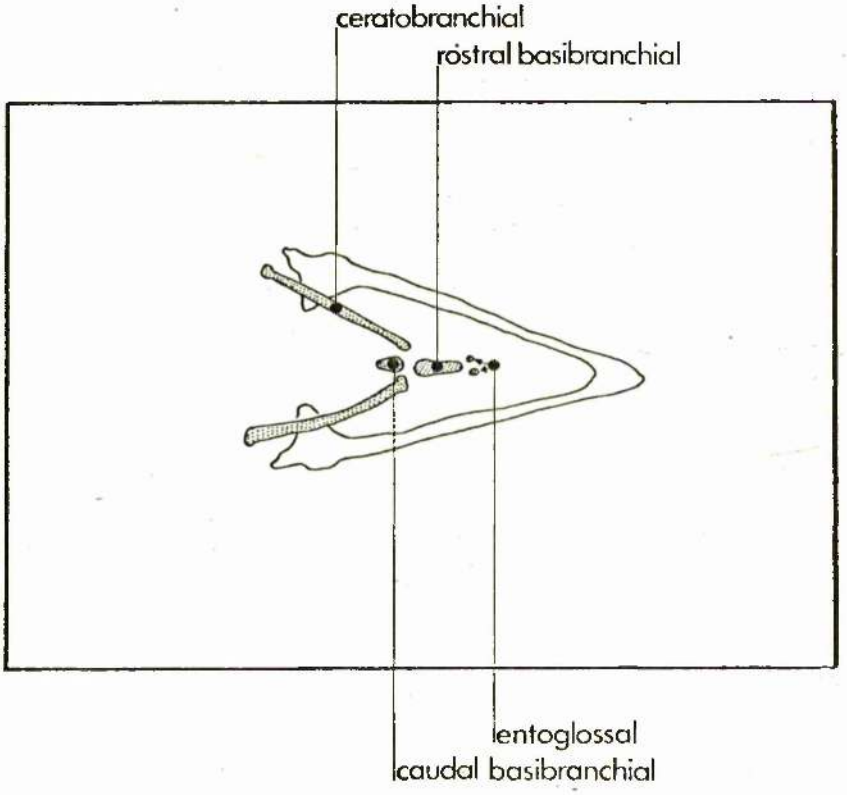
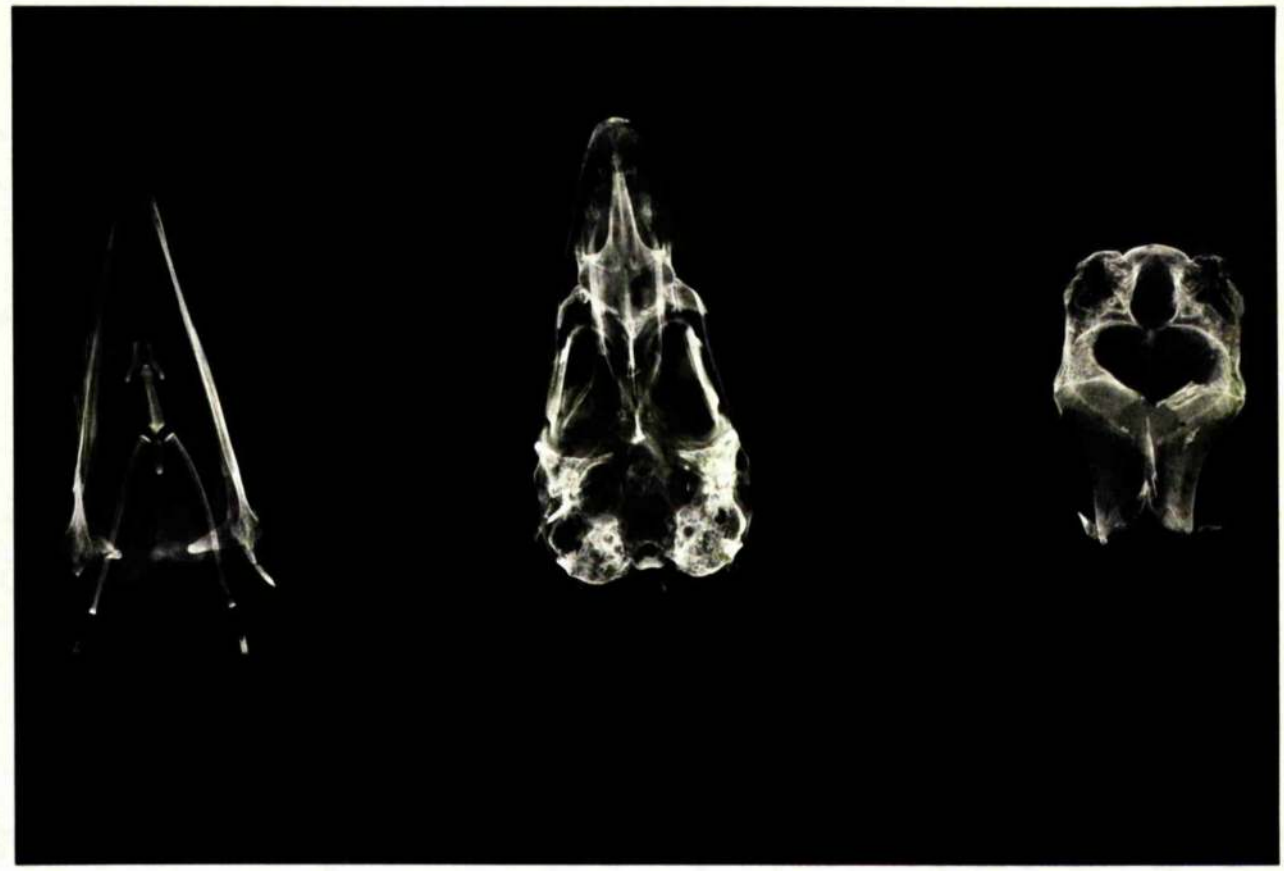


Fig.16 Mandible and hyoid 77 days
Ventral view
Alizarin



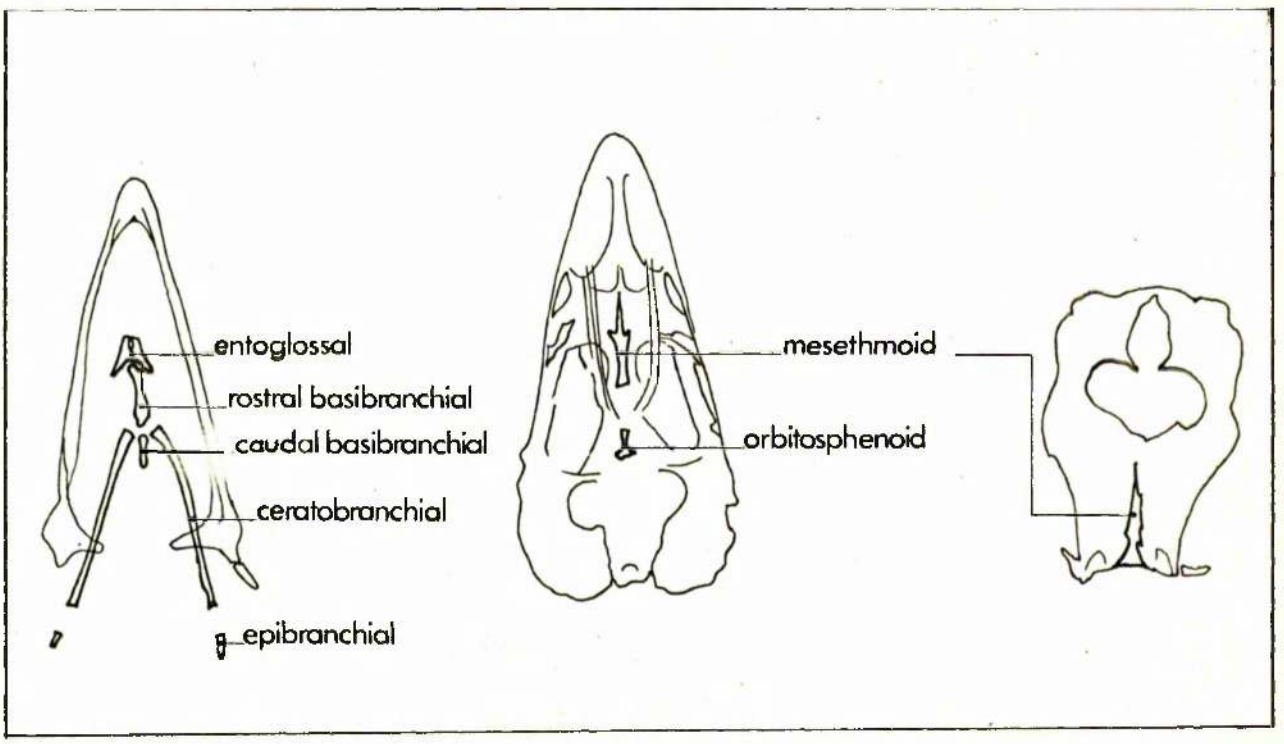
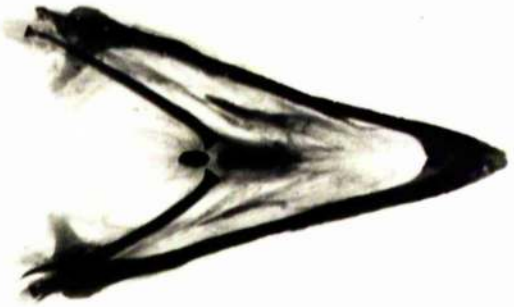


Fig. 17 Skull 91 days

Dorsoventral views:
Left mandible and hyoid
Middle floor of cranium and facial skeleton
Right roof of cranium

Silver nitrate/radiography



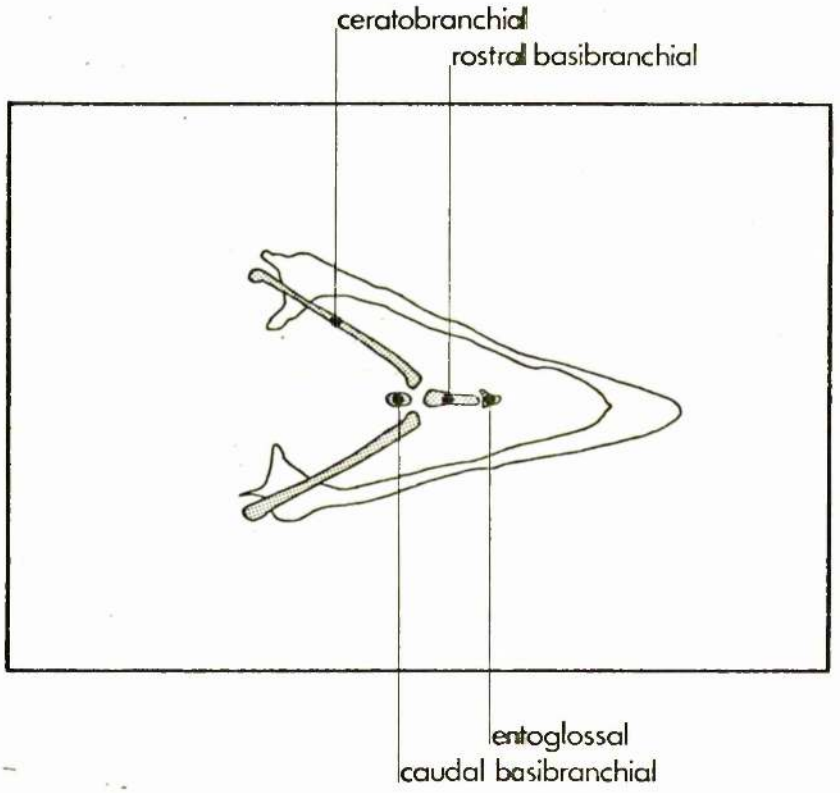


Fig. 18 Mandible and hyoid 98 days
Ventral view
Alizarin

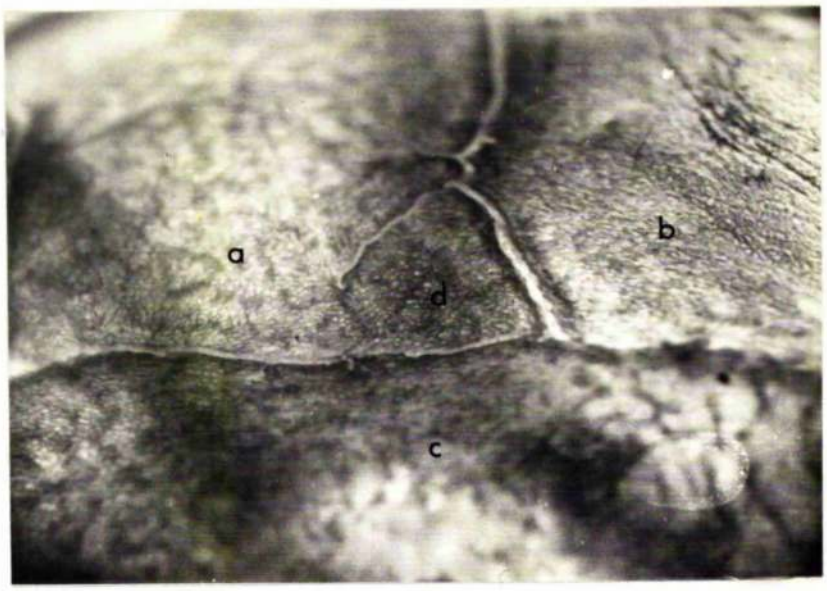


Fig. 19 Skull 35 days

Left lateral view

a. frontal

b. parietal

c. squamosal

d. sutural bone almost enclosed by surrounding bones

Alizarin

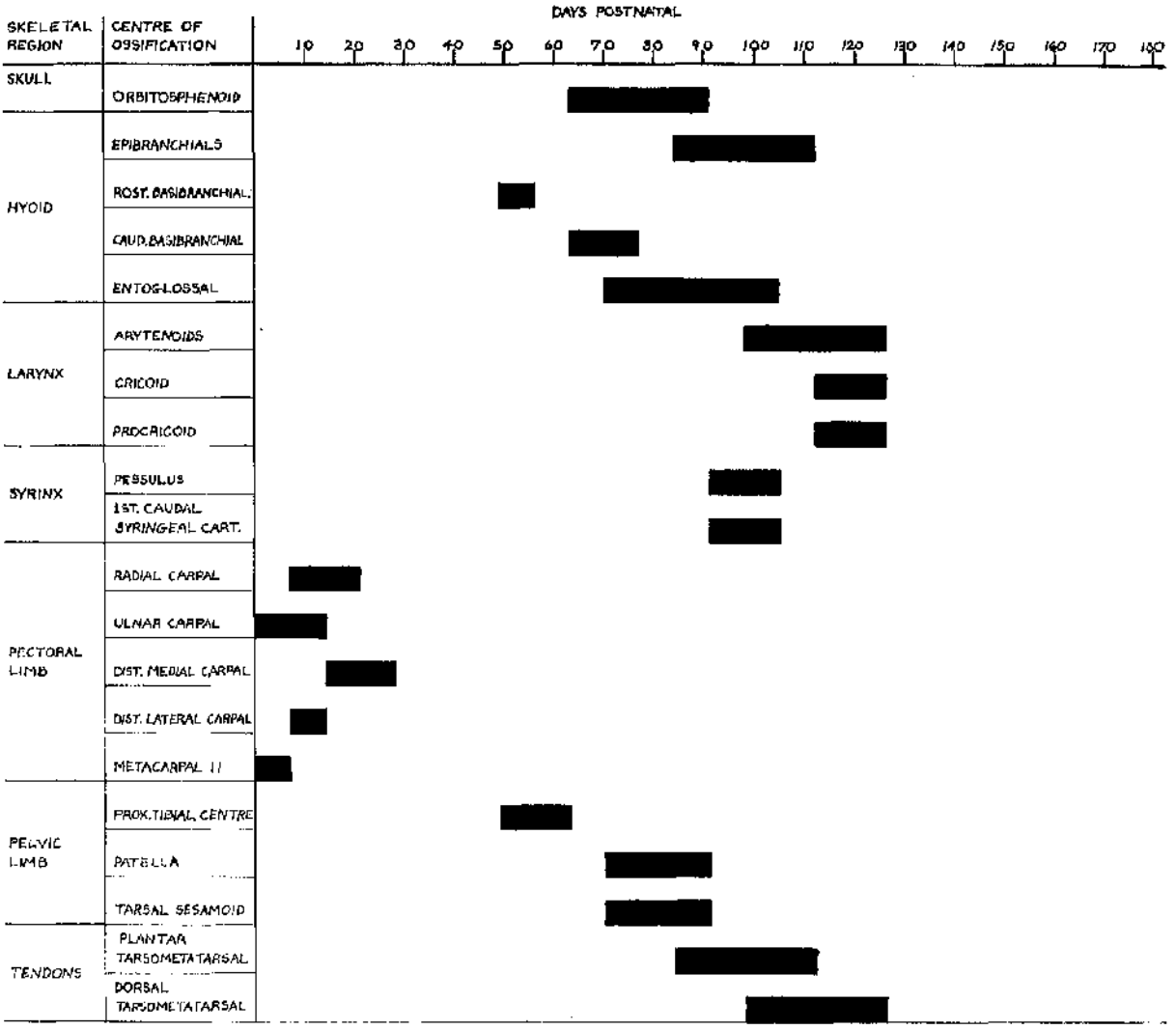


Fig. 20a Range of time of appearance of postnatally developing centres of ossification

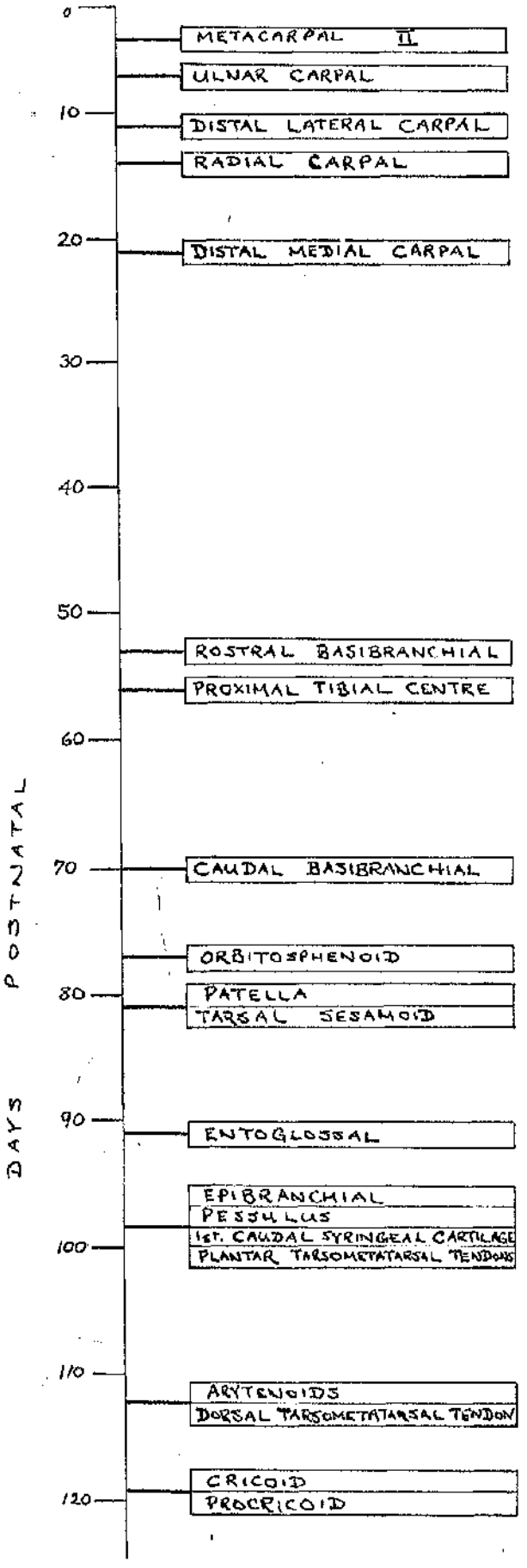


Fig. 20b Sequence of mean times of appearance of postnatally developing centres of ossification

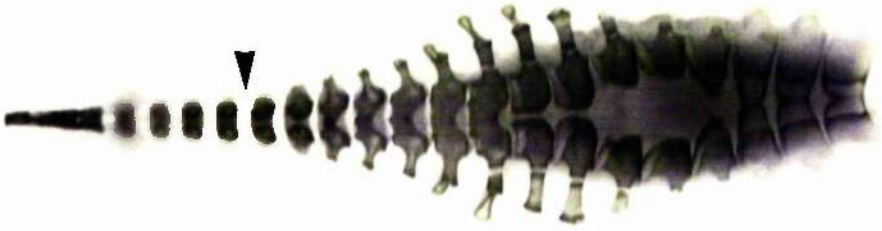


Fig. 21 Lumbosacral and coccygeal vertebrae 0 days
Dorsal view
arrow indicates lumbosacral/coccygeal junction
Alizarin

Fig. 22 Lumbosacral and coccygeal vertebrae 0 days
Ventral view
arrow indicates lumbosacral/coccygeal junction
Alizarin

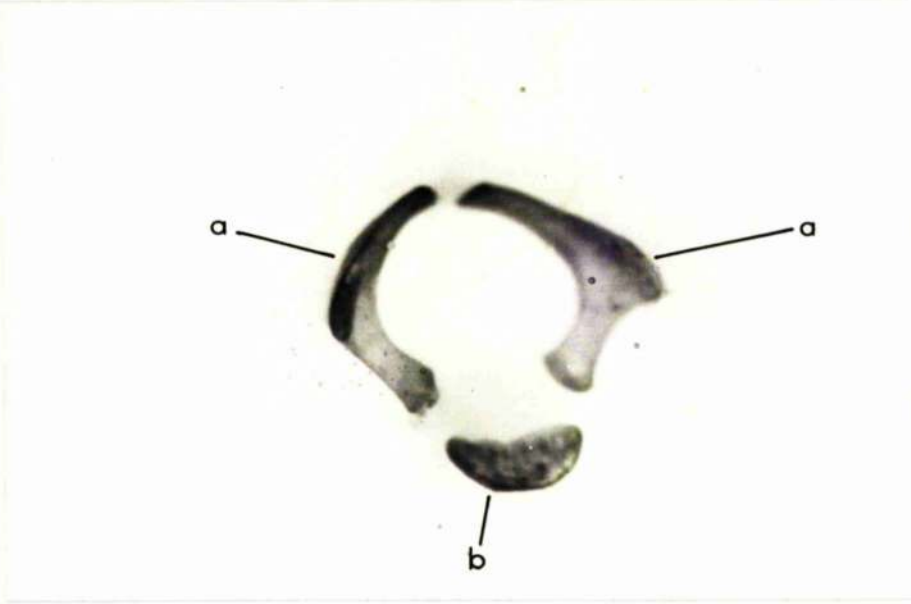


Fig. 23 Atlas 0 days

Cranial view

a. neural arch centres

b. $\overset{\text{inter}}{\wedge}$ centrum

Alizarin

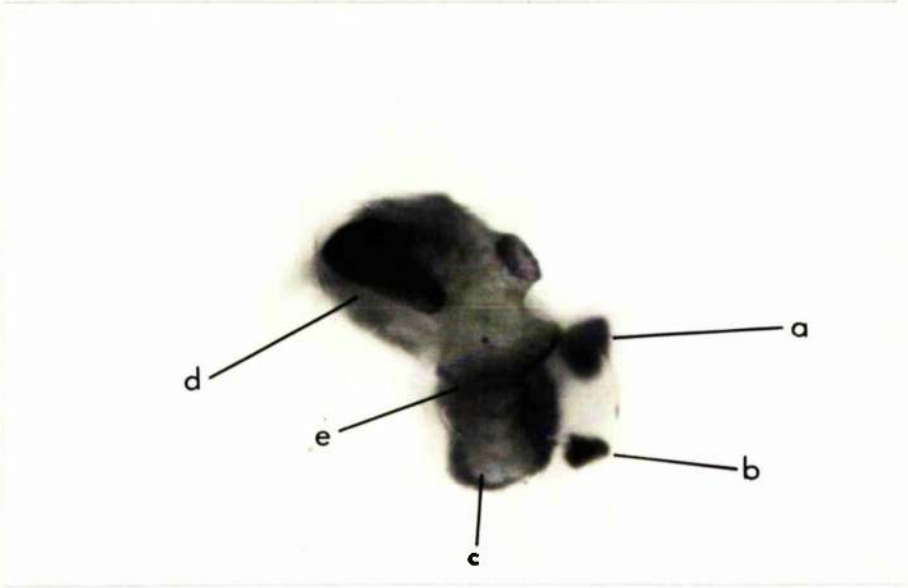


Fig. 24 Axis 0 days

Lateral view

a. odontoid process
b. intercentrum
c. centrum
d. neural arch
e. synchondrosis between centrum and neural arch

Alizarin

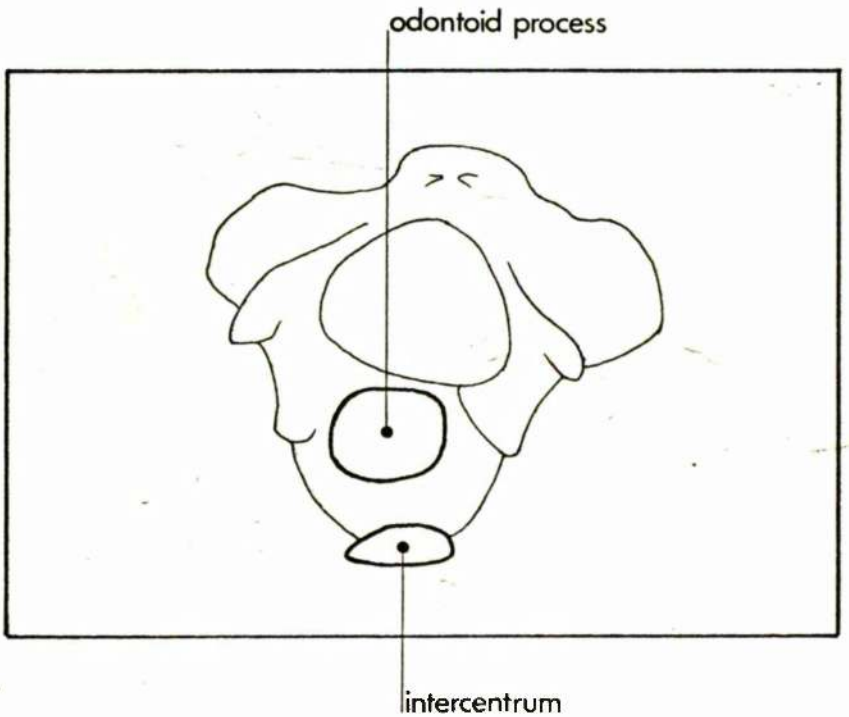
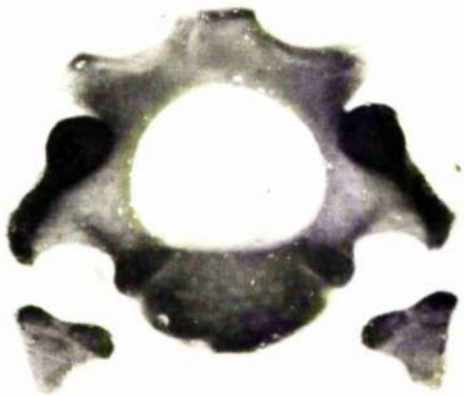


Fig. 25 Axis 0 days
Cranial view
Alizarin



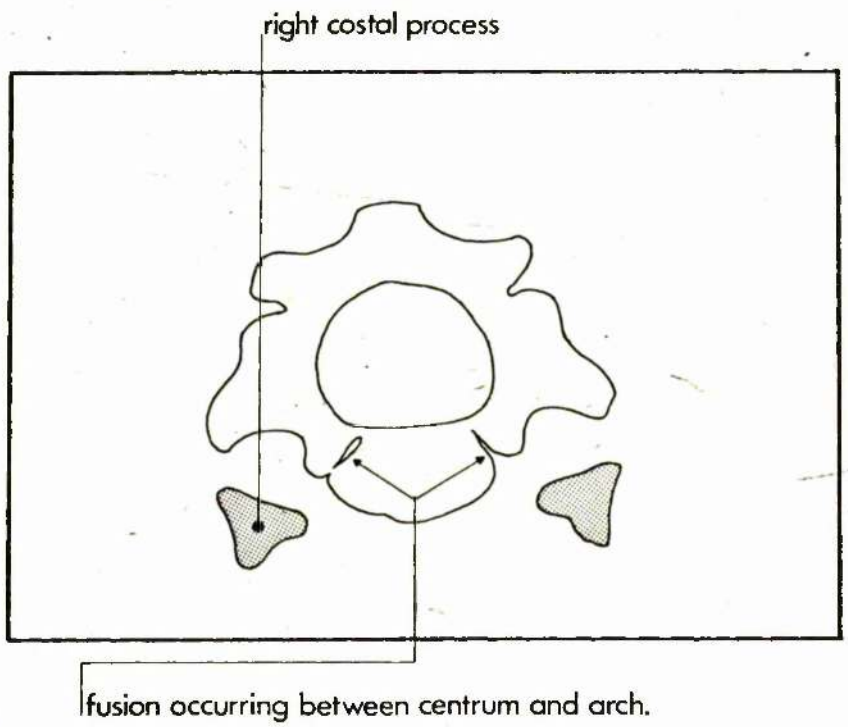


Fig. 26a 13th cervical vertebra 0 days
Cranial view
Alizarin

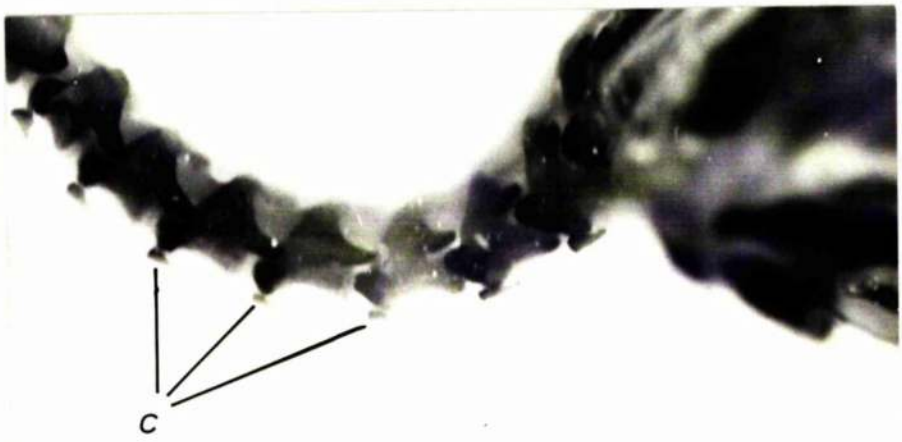


Fig. 26b Cervical vertebrae 0 days
Left view
c. costal elements
Alizarin

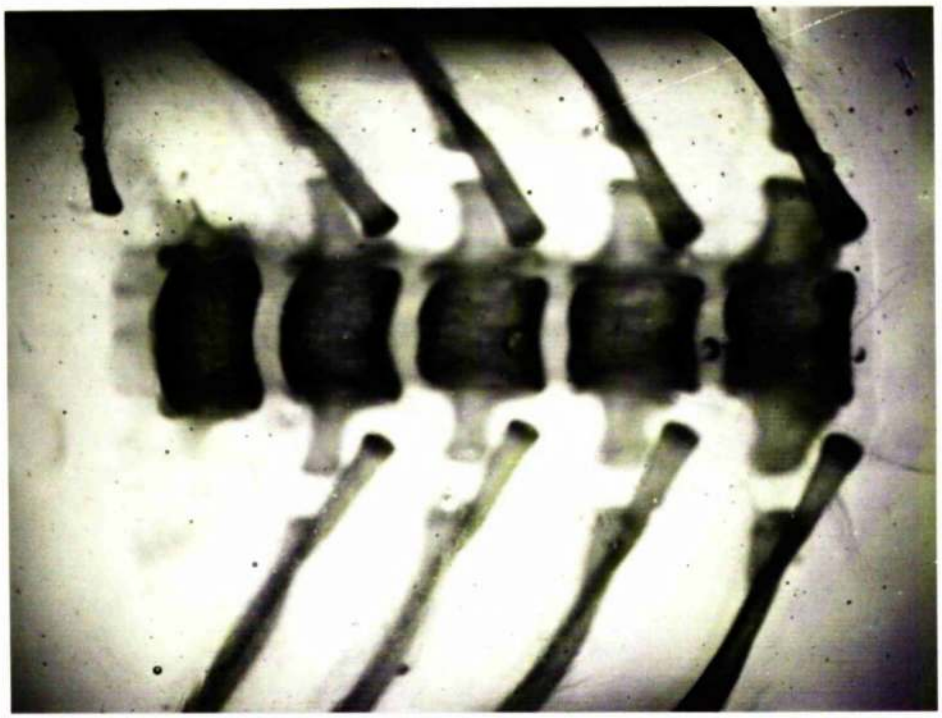


Fig. 27a Thoracic vertebrae and vertebral segments of ribs 0 days
Ventral view

Alizarin

The right rib of T5 has been lost and the left displaced caudad in the preparation of the specimen

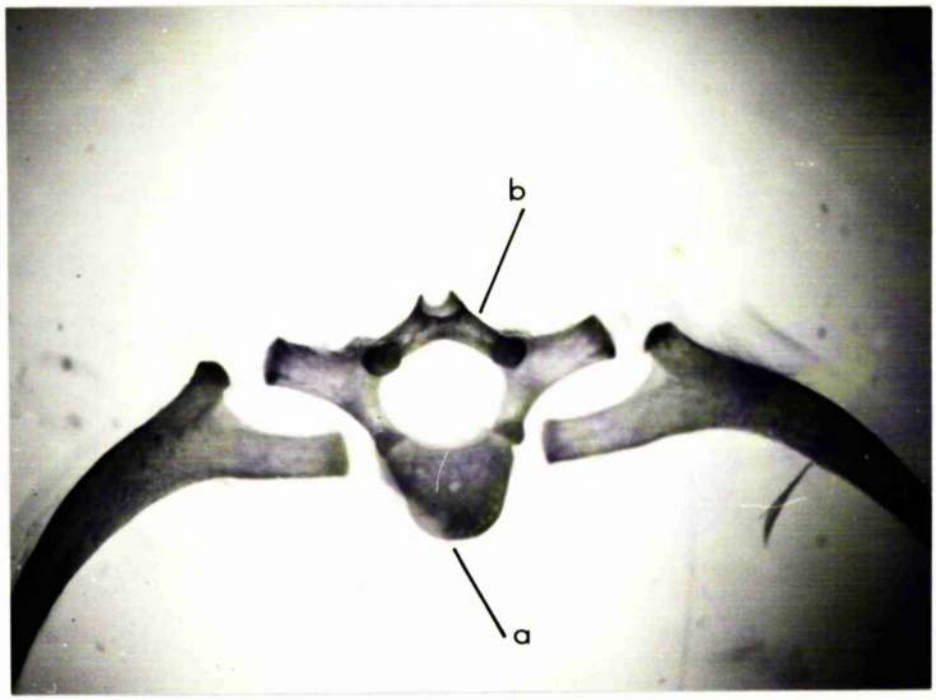


Fig. 27b Thoracic vertebra 1 and vertebral segments of ribs 0 days

Cranial view

a. centrum

b. single centre for neural arch

Alizarin

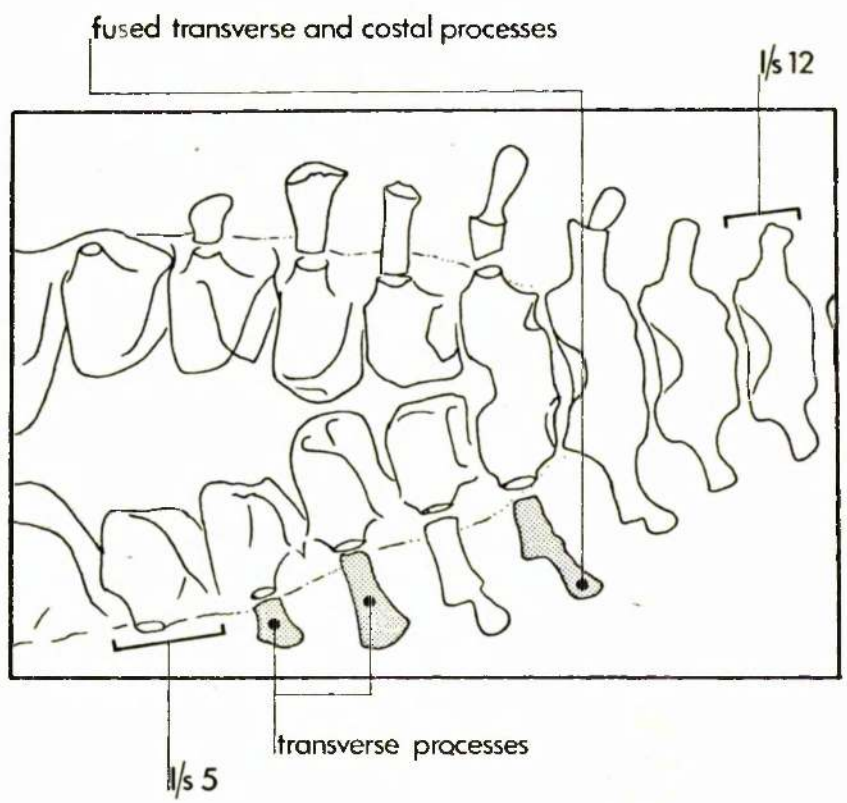


Fig. 28 Lumbosacral vertebrae 5-12 0 days
Dorsal view
Alizarin

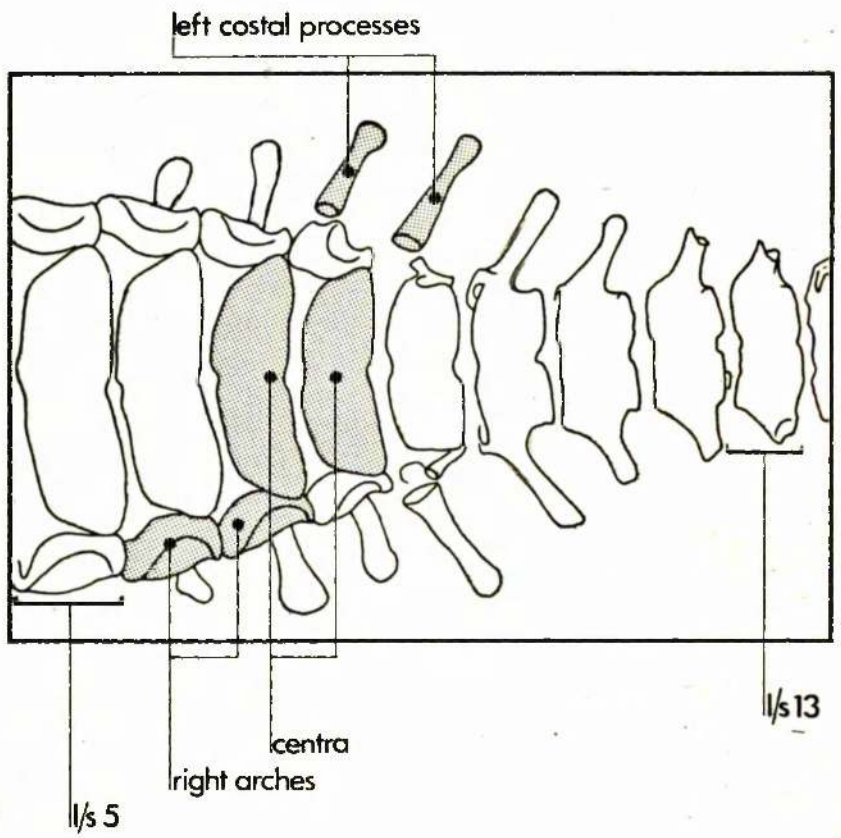


Fig. 29 Lumbosacral vertebrae 5-13 0 days
Ventral view
Alizarin

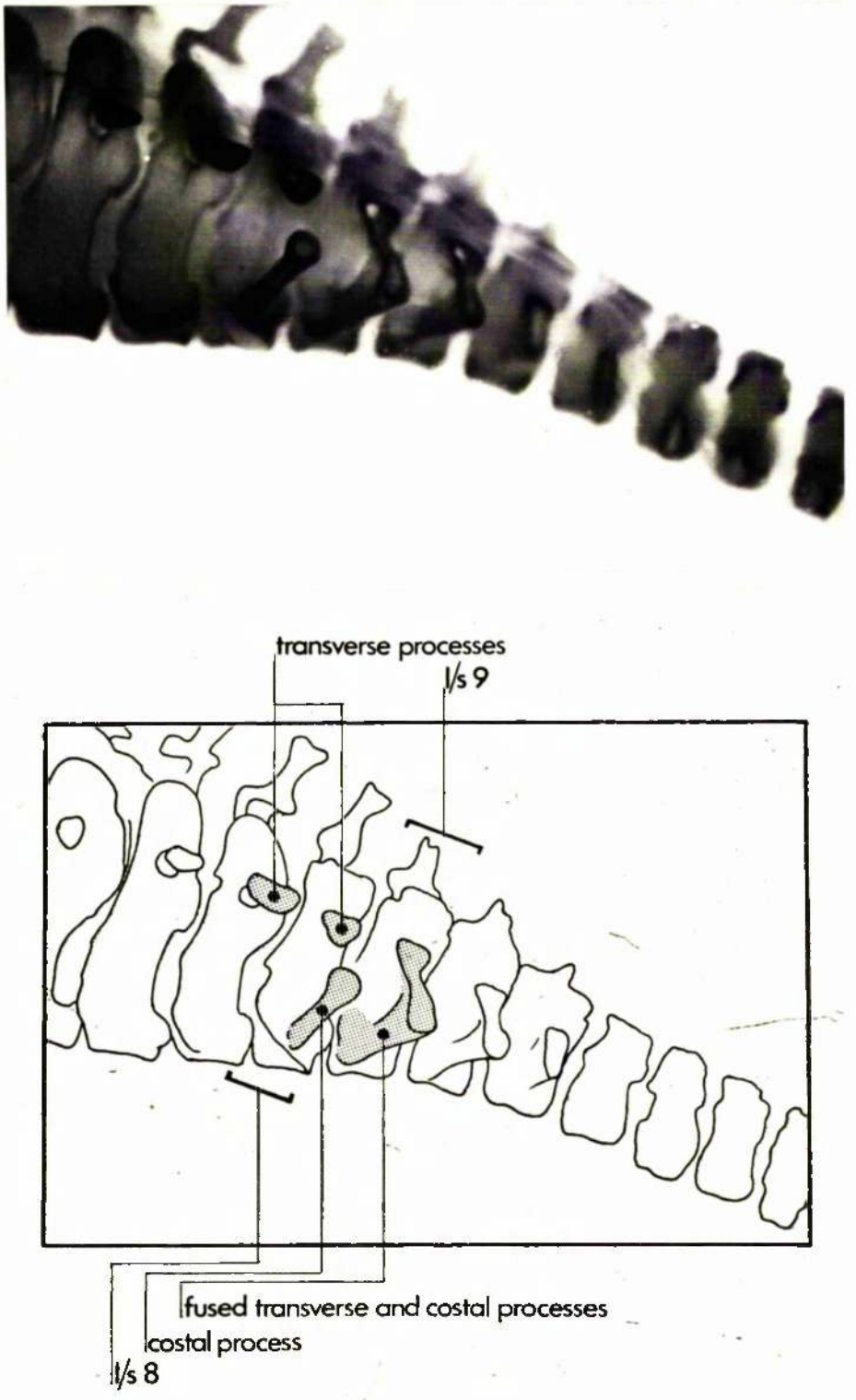


Fig. 30 Lumbosacral vertebrae 6-14 0 days
Lateral view
Alizarin

Fig. 31 Coccygeal vertebrae 0 days
Left lateral view
Alizarin

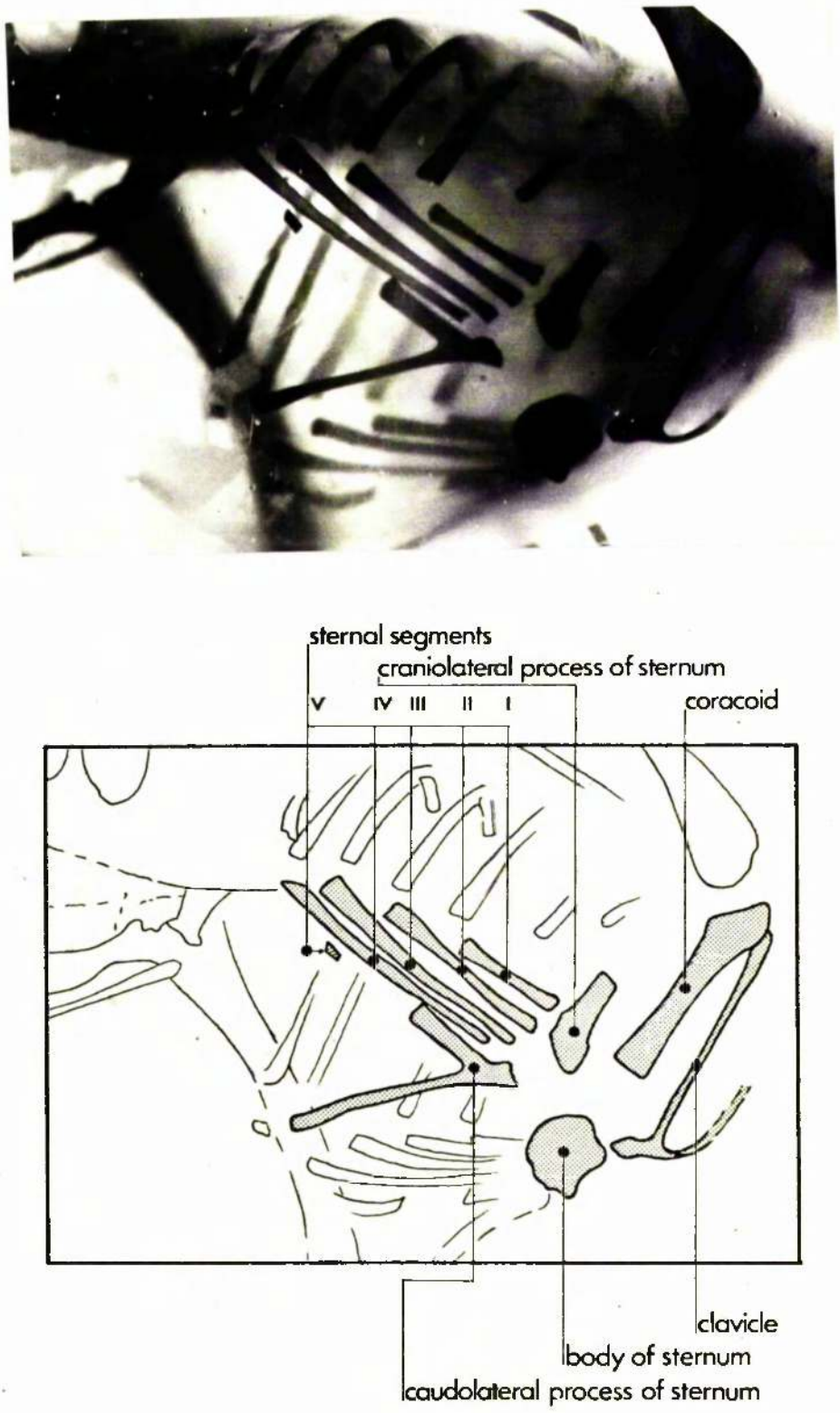
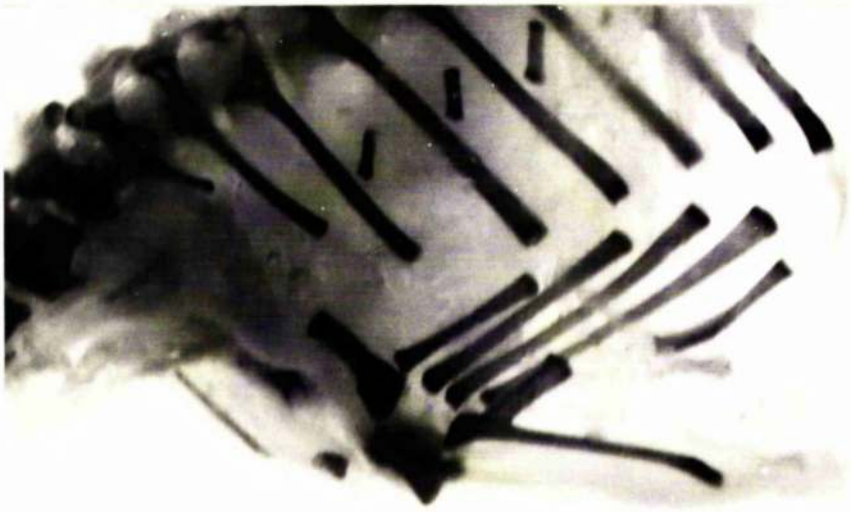


Fig. 32 Sternum and sternal ribs 0 days
Right lateral view
Alizarin



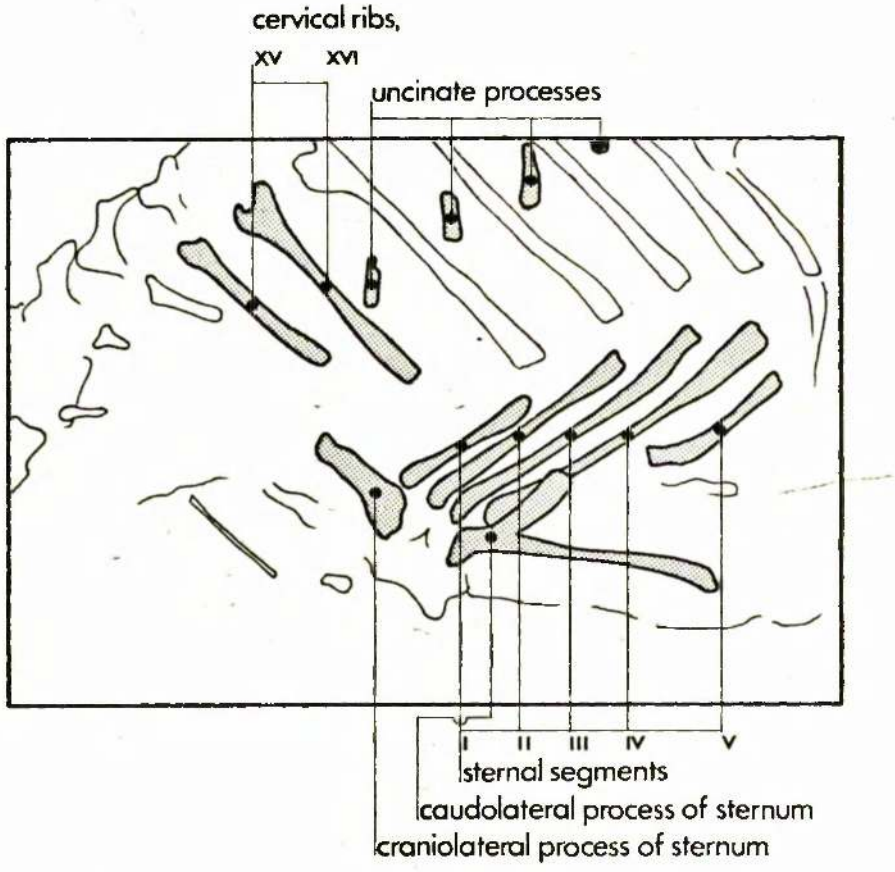
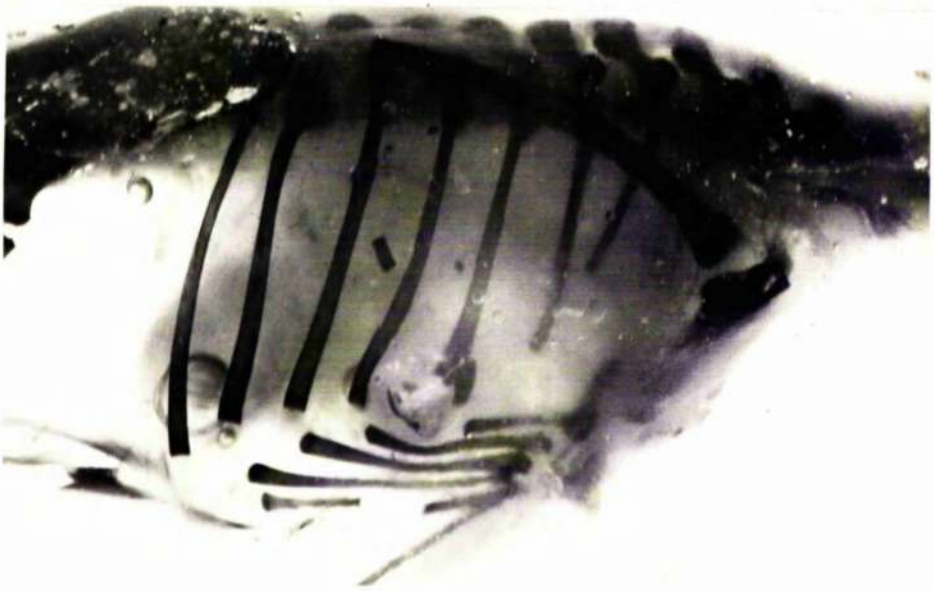


Fig. 33 Ribs and sternum
Left lateral view
Alizarin

0 days



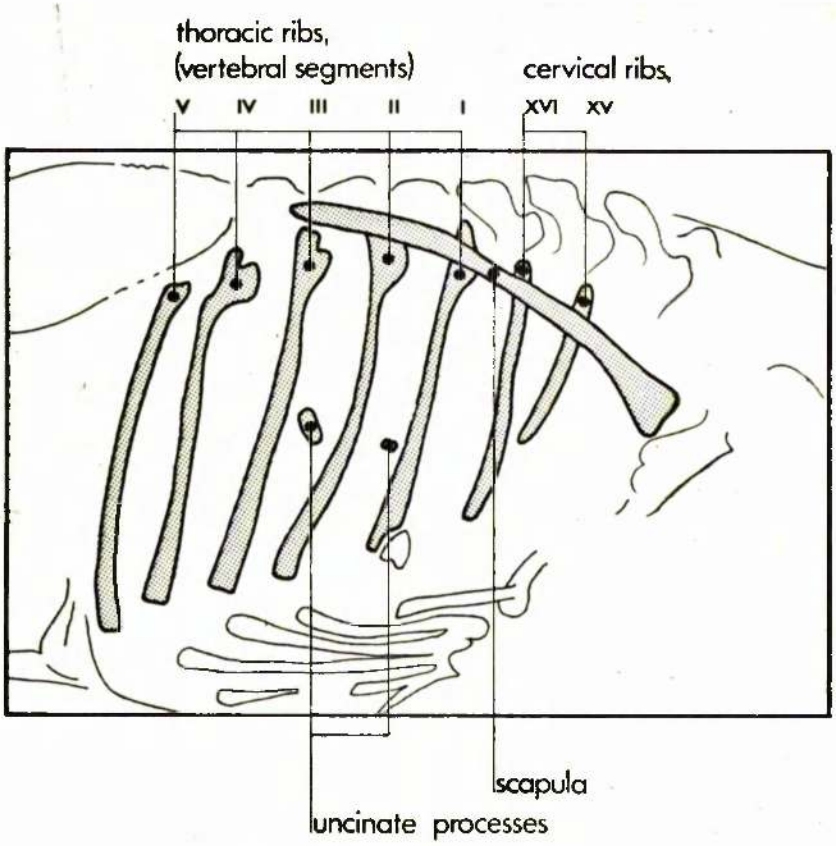


Fig. 34 Vertebral ribs 0 days
Right lateral view
Alizarin

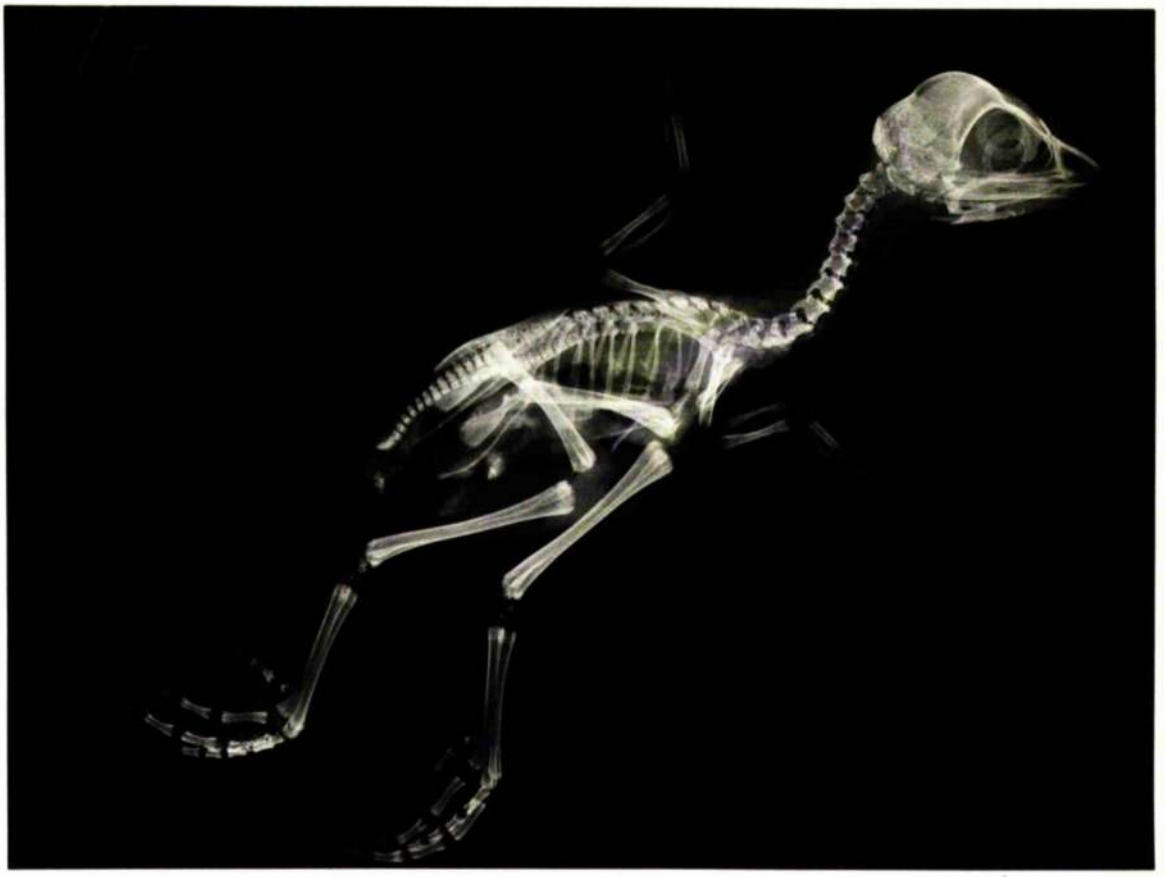


Fig. 35 Whole skeleton 0 days
Lateral view
Silver nitrate/radiography

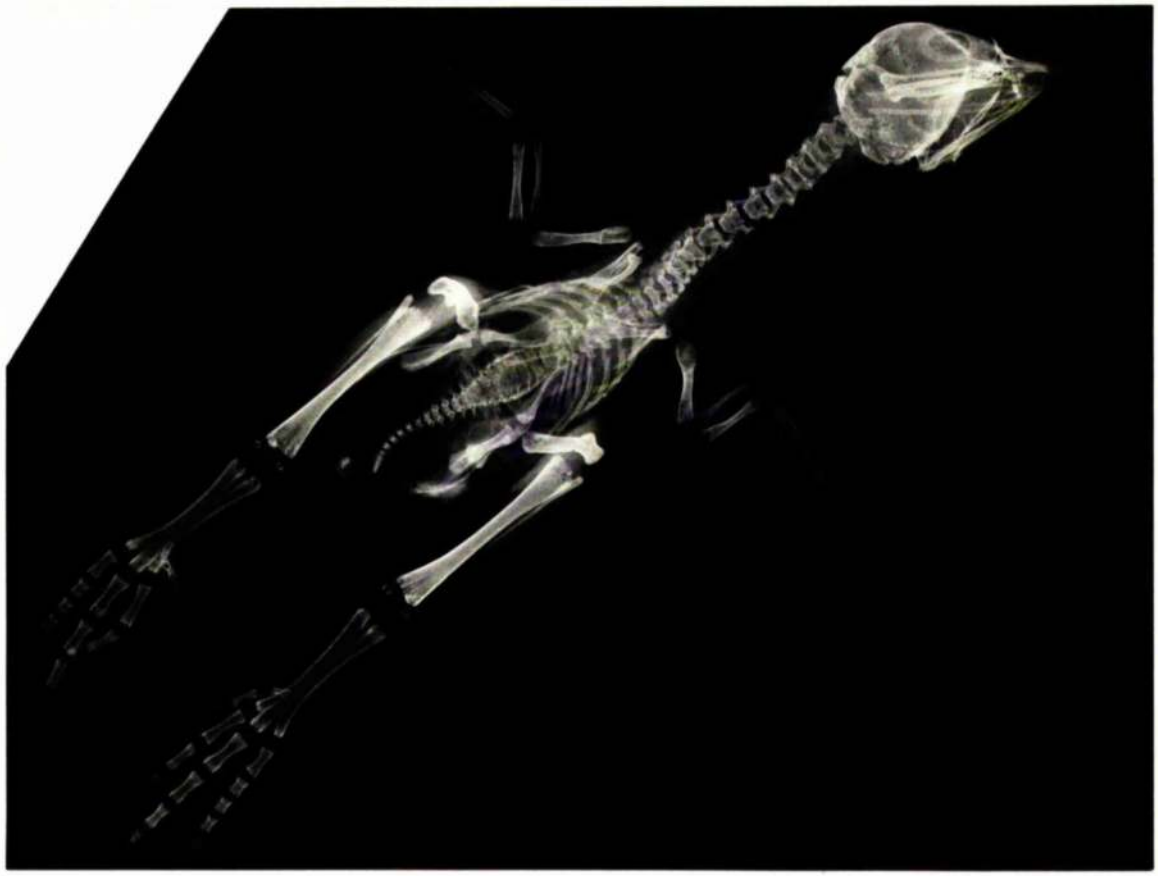


Fig. 36 Whole skeleton 0 days
Dorsoventral view
Silver nitrate/radiography

Fig. 37 Whole skeleton 0 days
Ventral view
Alizarin

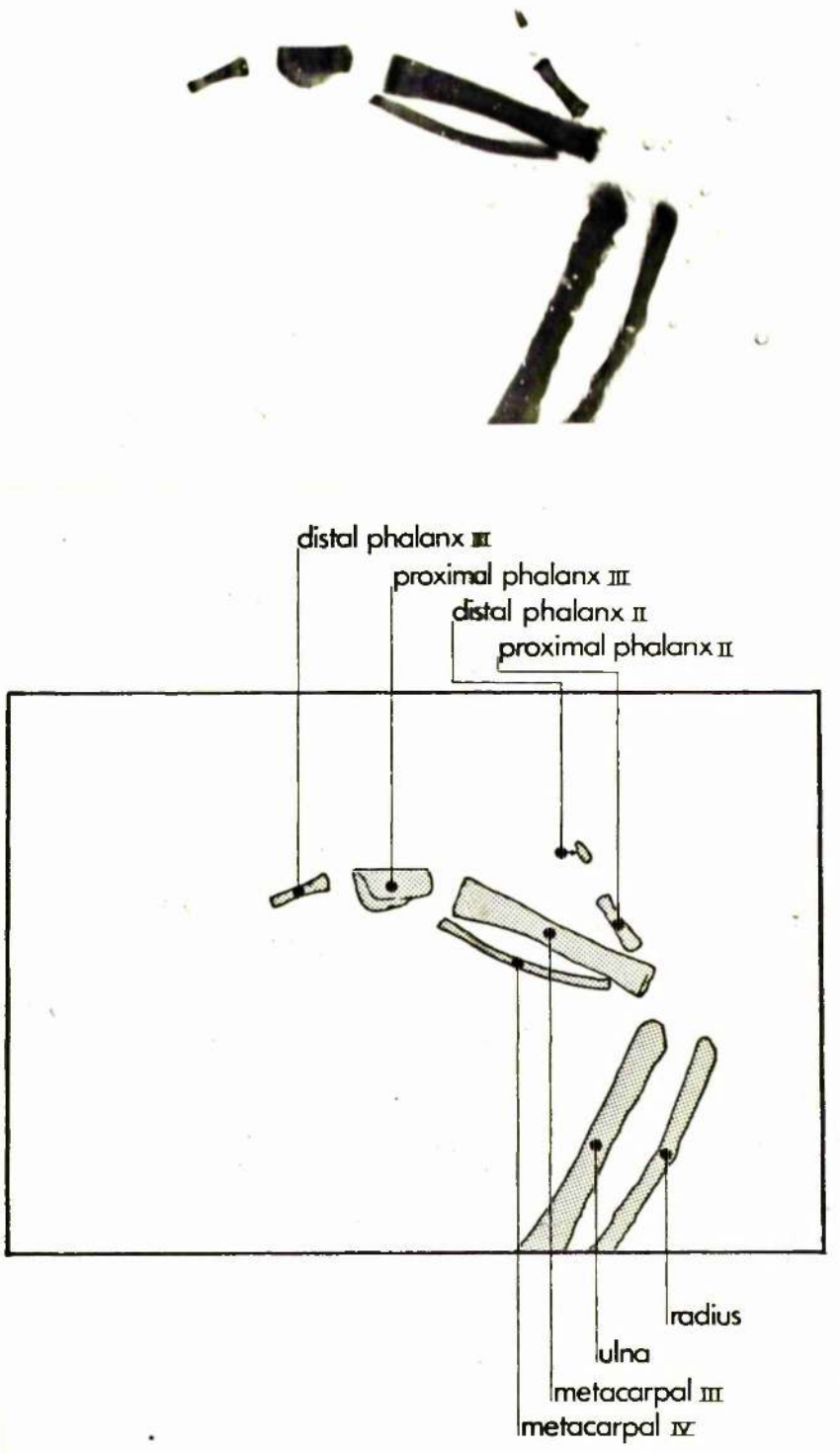


Fig. 38 Skeleton of right manus 0 days
Dorsal view
Alizarin

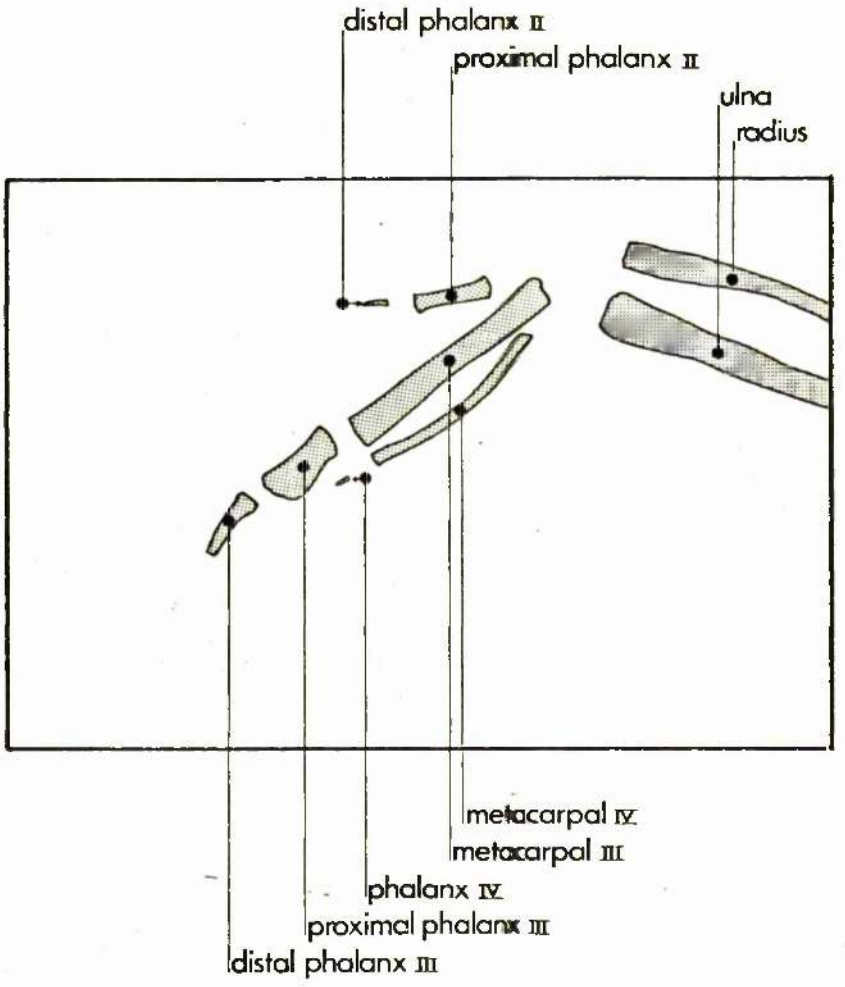


Fig. 39 Left manus 0 days
Dorsal view
Alizarin

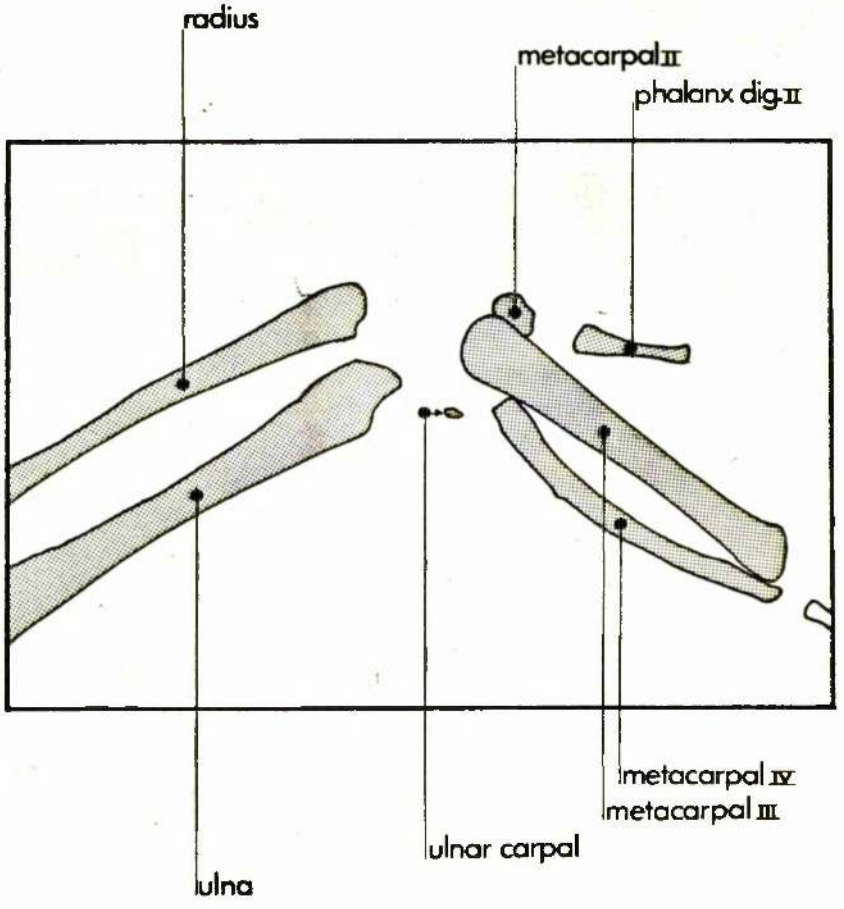
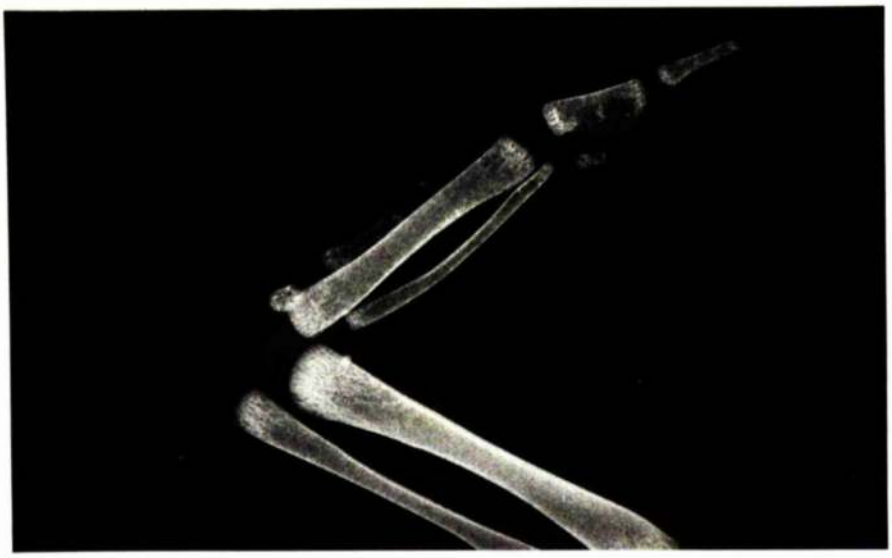


Fig. 40 Left carpus and incomplete manus 7 days
Palmar view
Alizarin



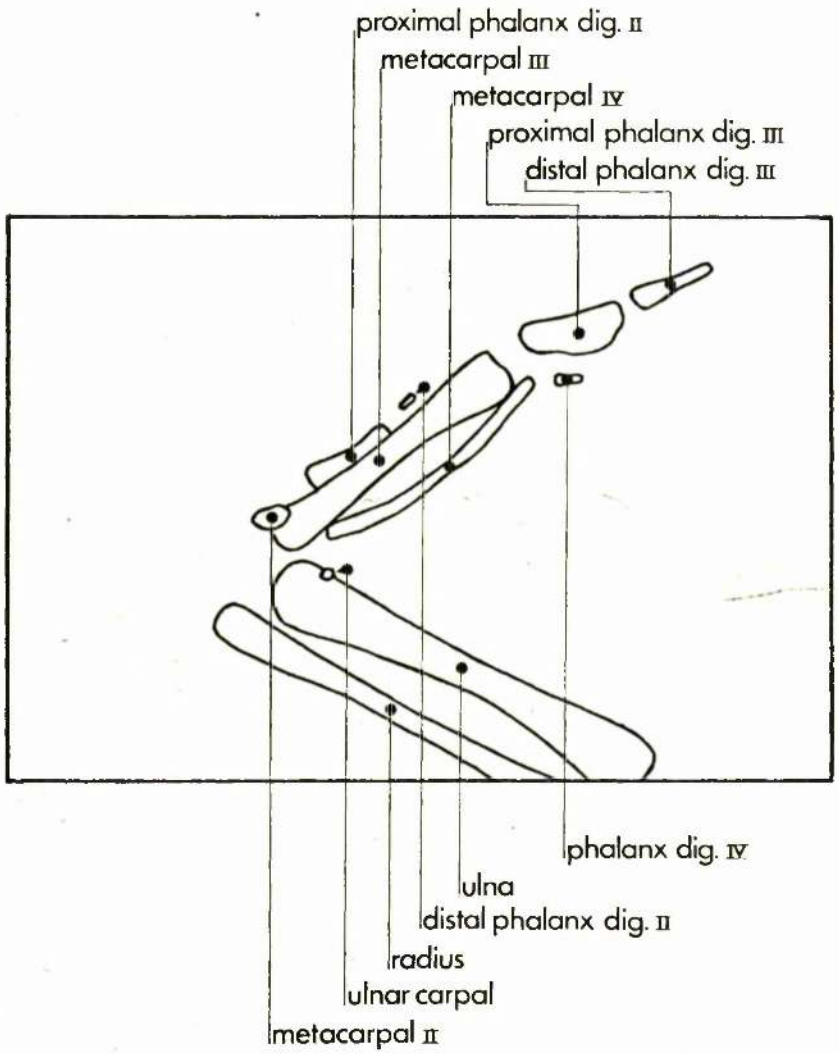


Fig. 41 Left carpus and manus 7 days
Dorsopalmar view
Silver/nitrate radiography

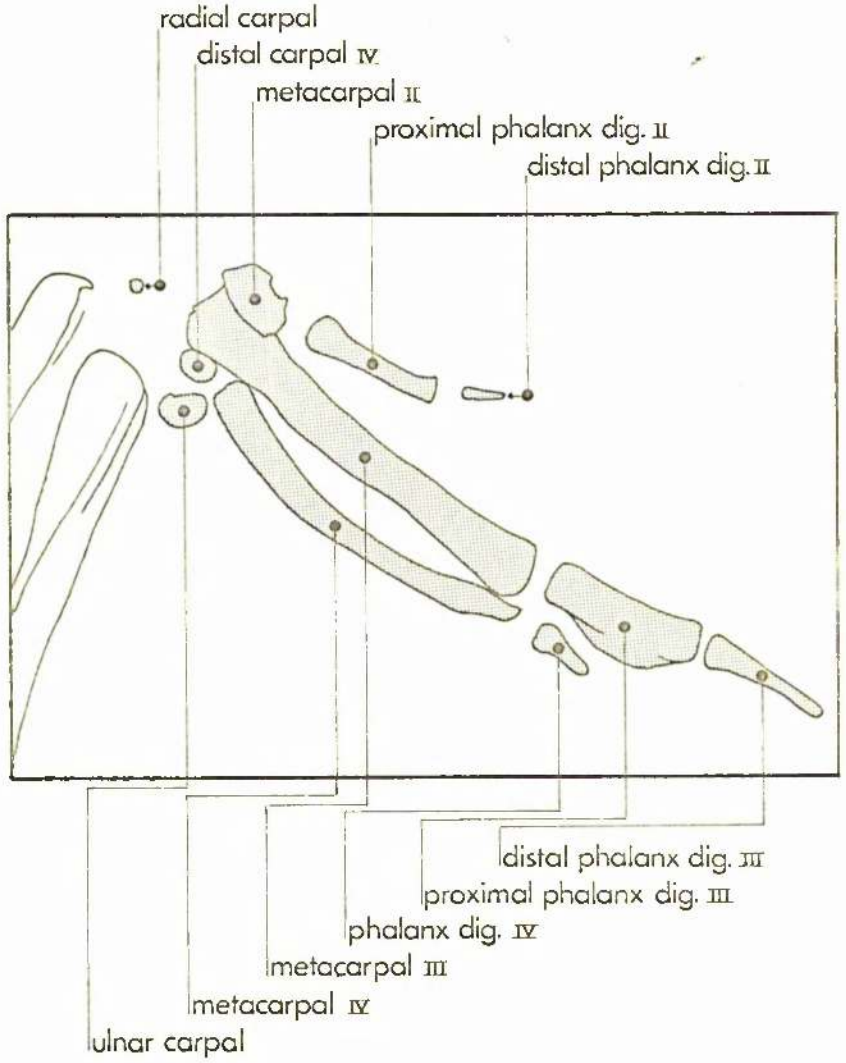
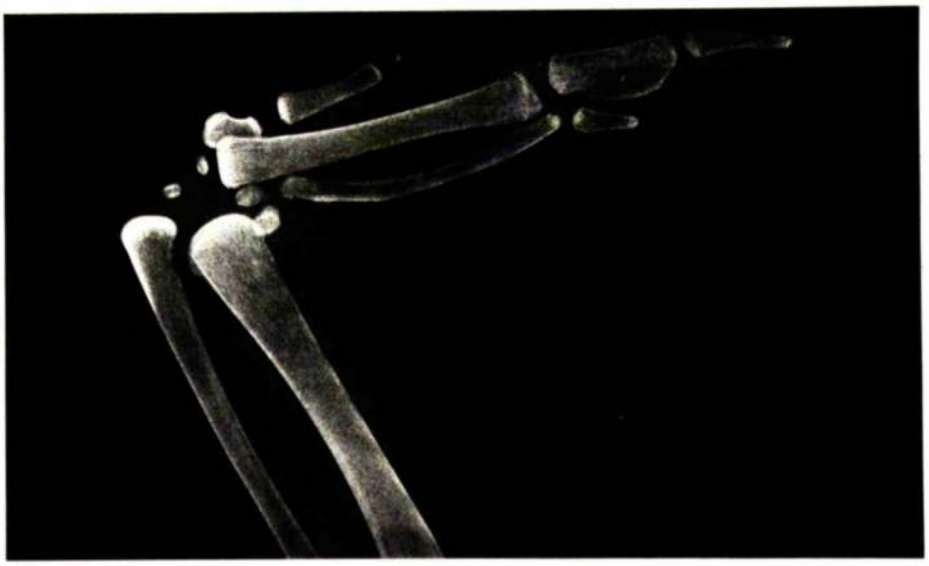


Fig.42 Left carpus and manus 14 days
Palmar view
Alizarin



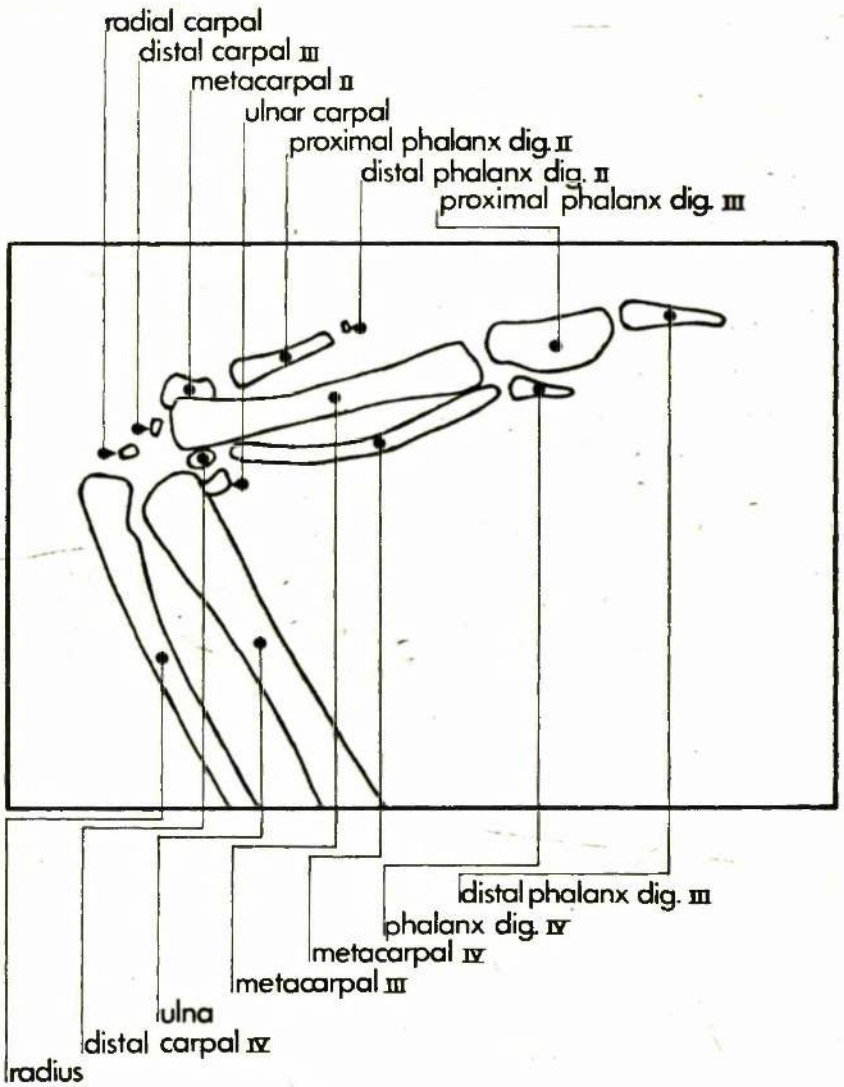


Fig. 43 Right carpus and manus 21 days
Dorsopalmar view
Silver nitrate/radiography

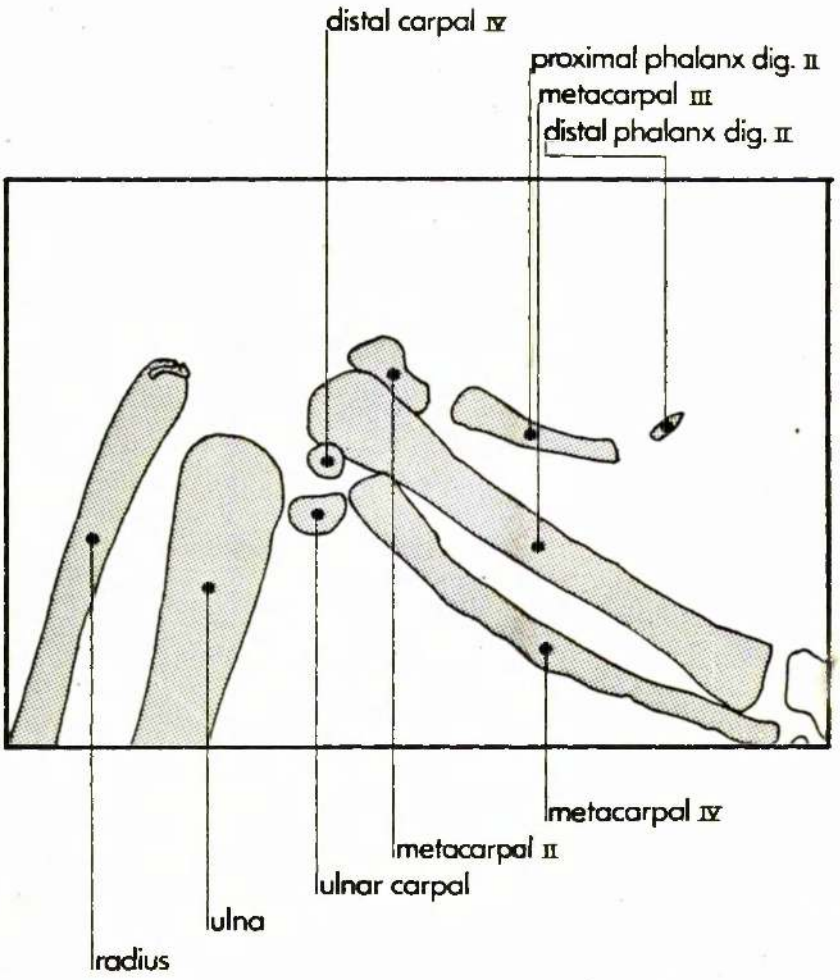


Fig. 44 Left carpus 21 days
Palmar view
Alizarin

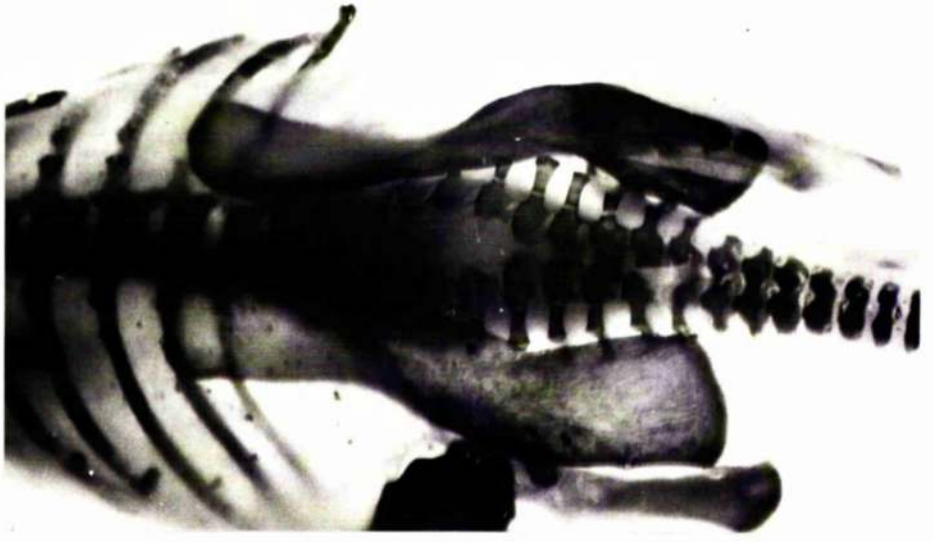
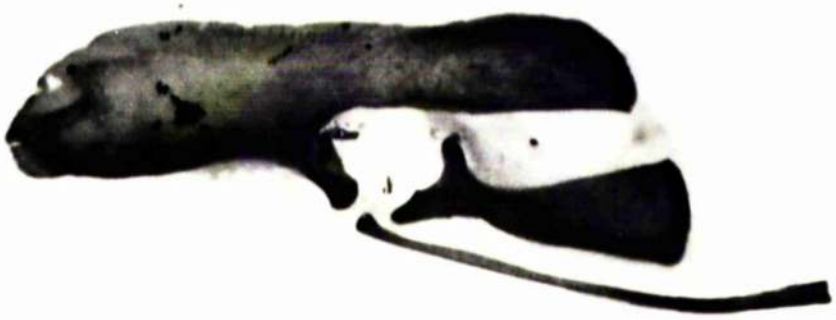


Fig. 45 Ilia, thoracic and lumbosacral vertebrae
0 days
Dorsal view
Alizarin



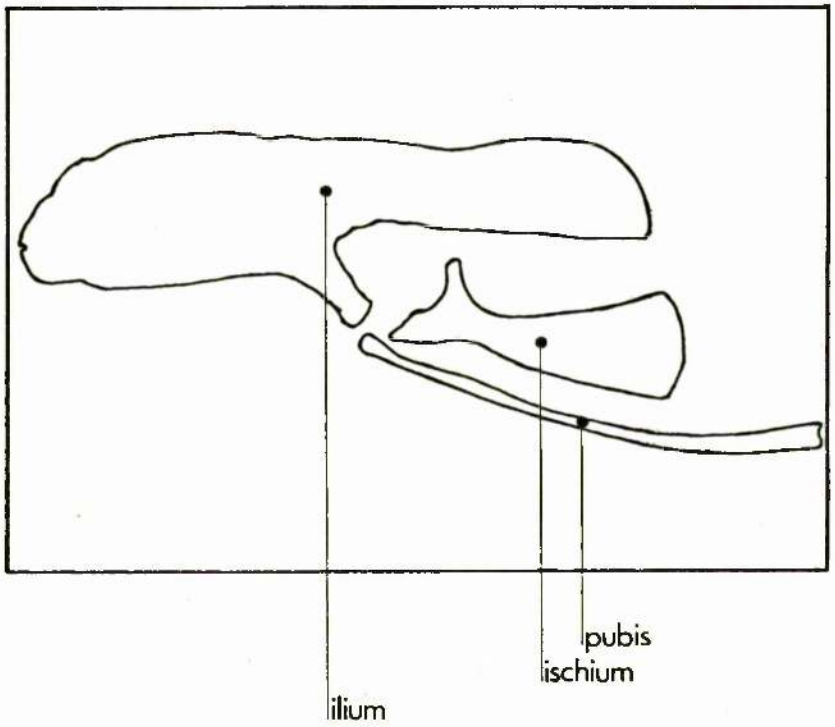


Fig. 46 Os coxae 0 days
Lateral view
Alizarin

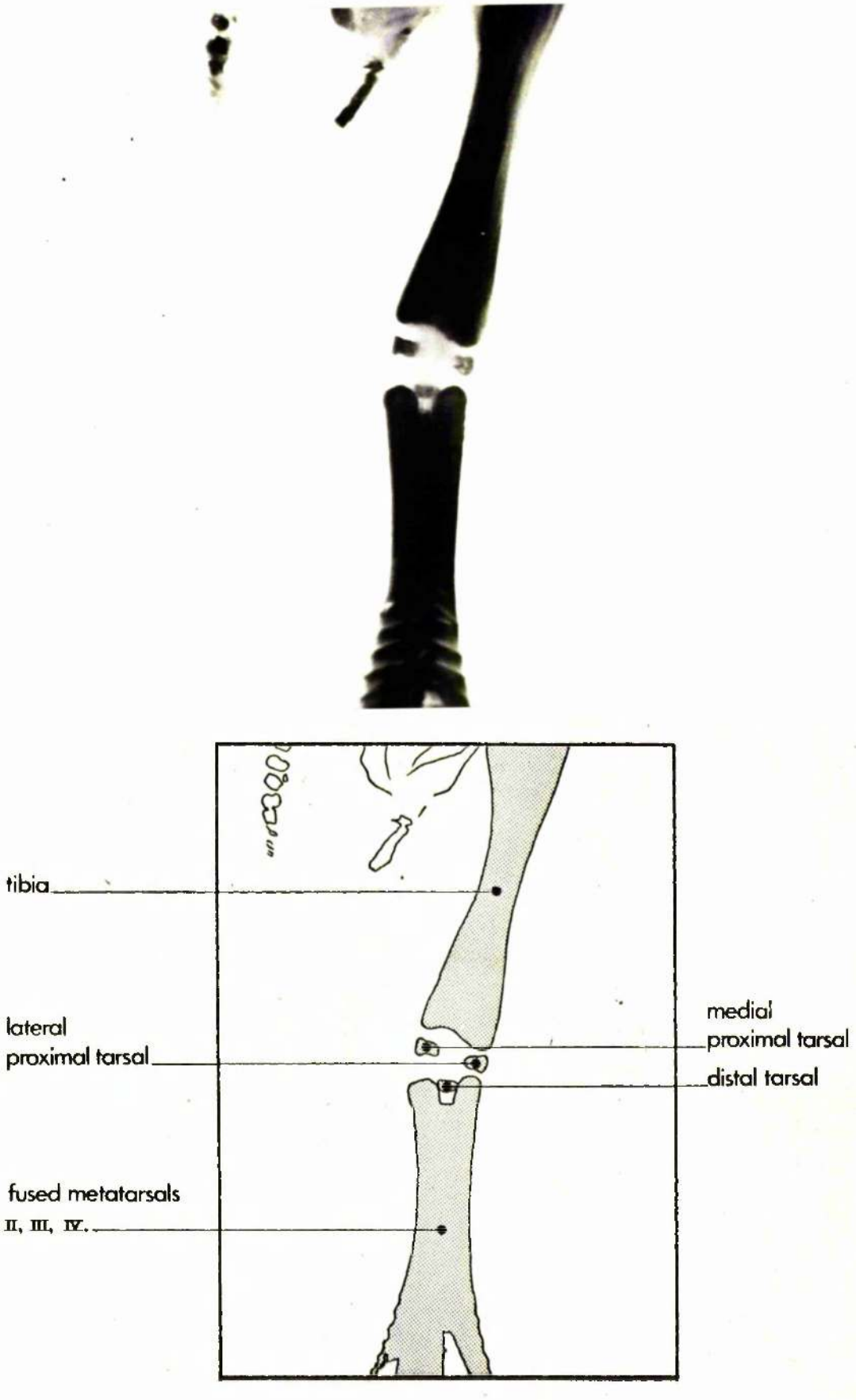


Fig. 47 Tarsus
Dorsal view
Alizarin
0 days

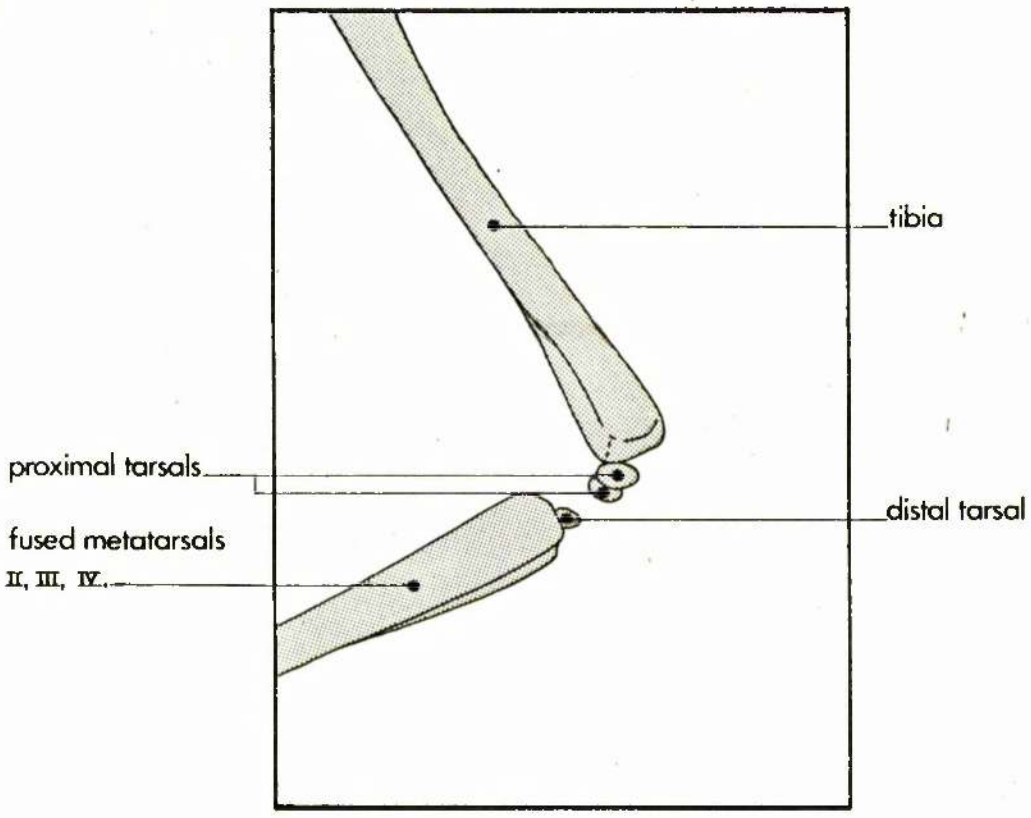


Fig. 48 Tarsus 0 days
Lateral view
Alizarin

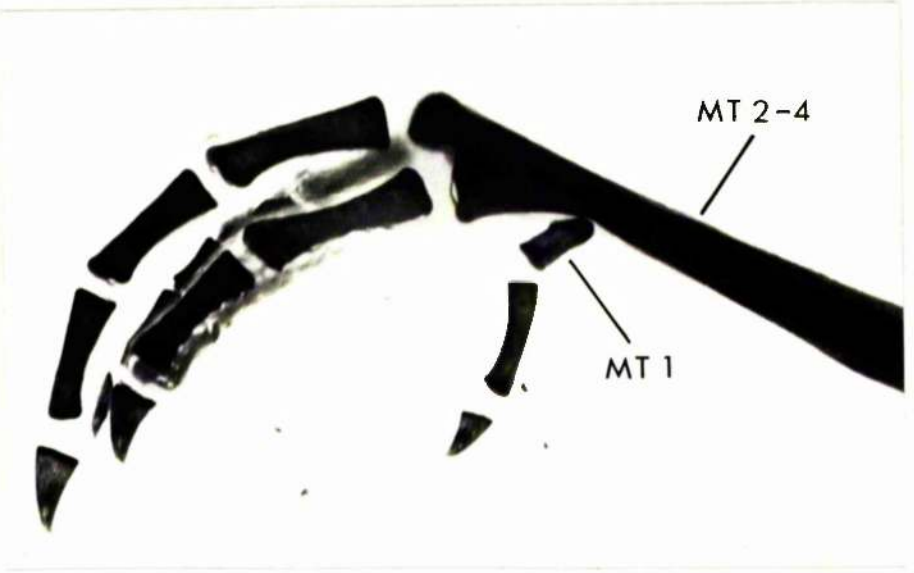


Fig. 49 Right pes 0 days
Medial view
MT1 1st metatarsal
MT2-4 fused metatarsals 2-4
Alizarin

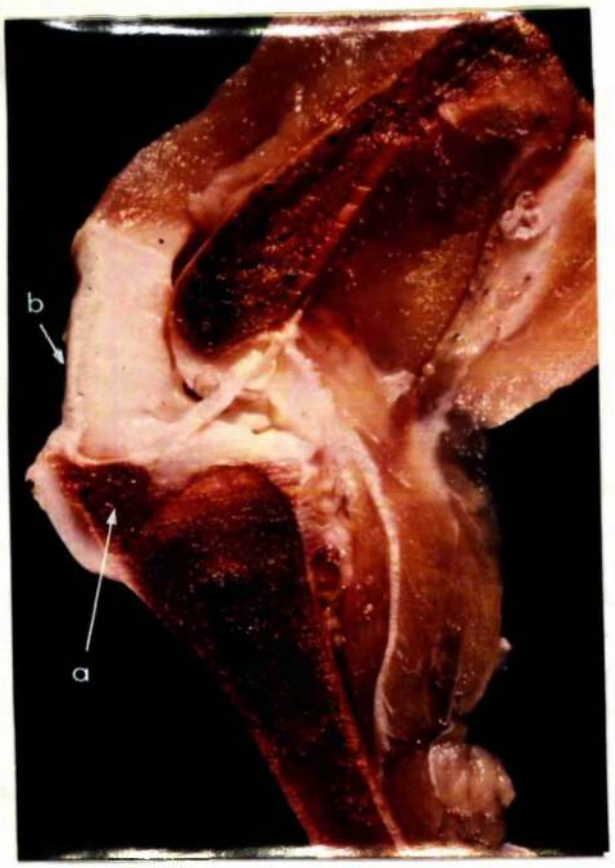


Fig. 50a
Stifle joint fowl 70days
Midsagittal section
a. prox. tibial centre
b. patellar ligament

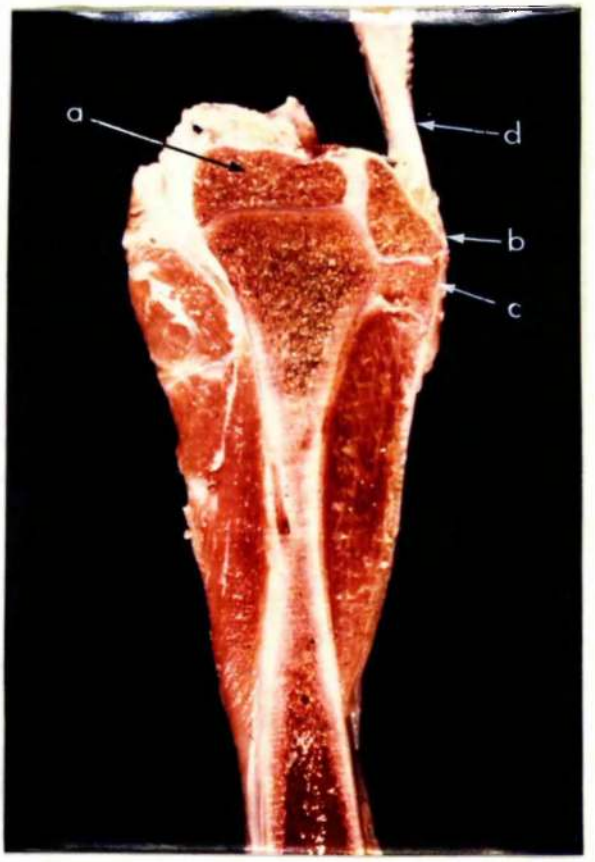


Fig. 50b
Stifle joint pup 5months
Midsagittal section
a. prox. 'pressure' epiphysis
b. 'traction' epiphysis
c. tibial crest apophysis
d. patellar ligament

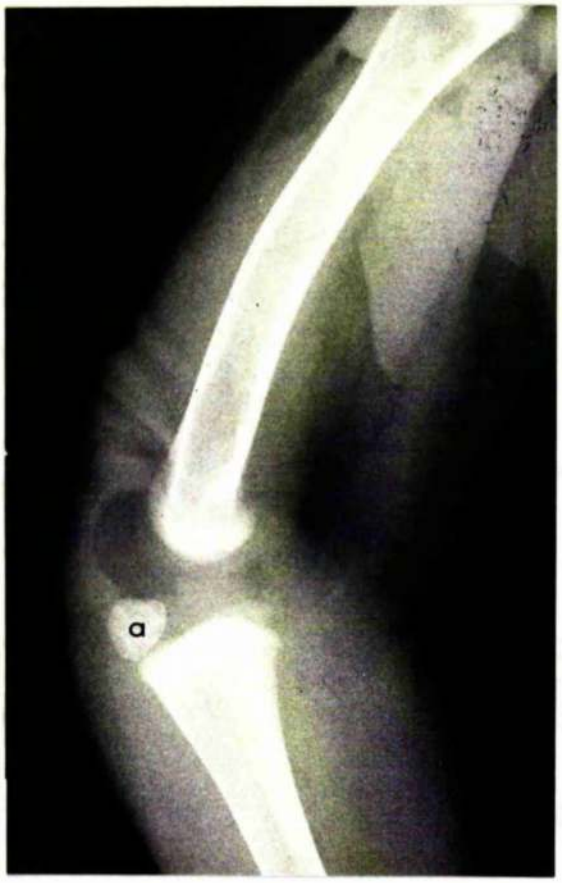


Fig. 50c
Stifle joint fowl 70 days
Lateral view
a. prox. tibial centre
Radiography

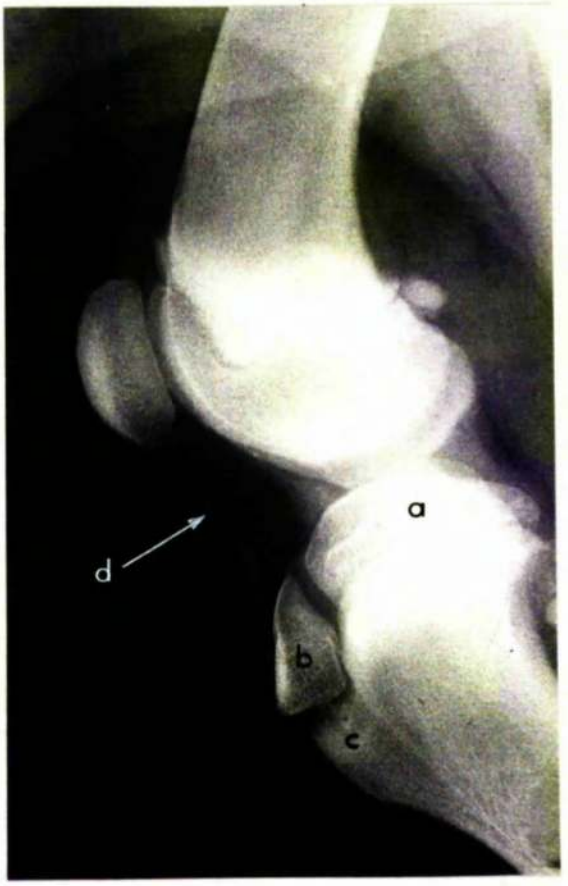


Fig. 50d
Stifle joint pup 5 months
Lateral view
a. prox. 'pressure' epiphysis
b. 'traction' epiphysis
c. tibial crest apophysis
d. patellar ligament

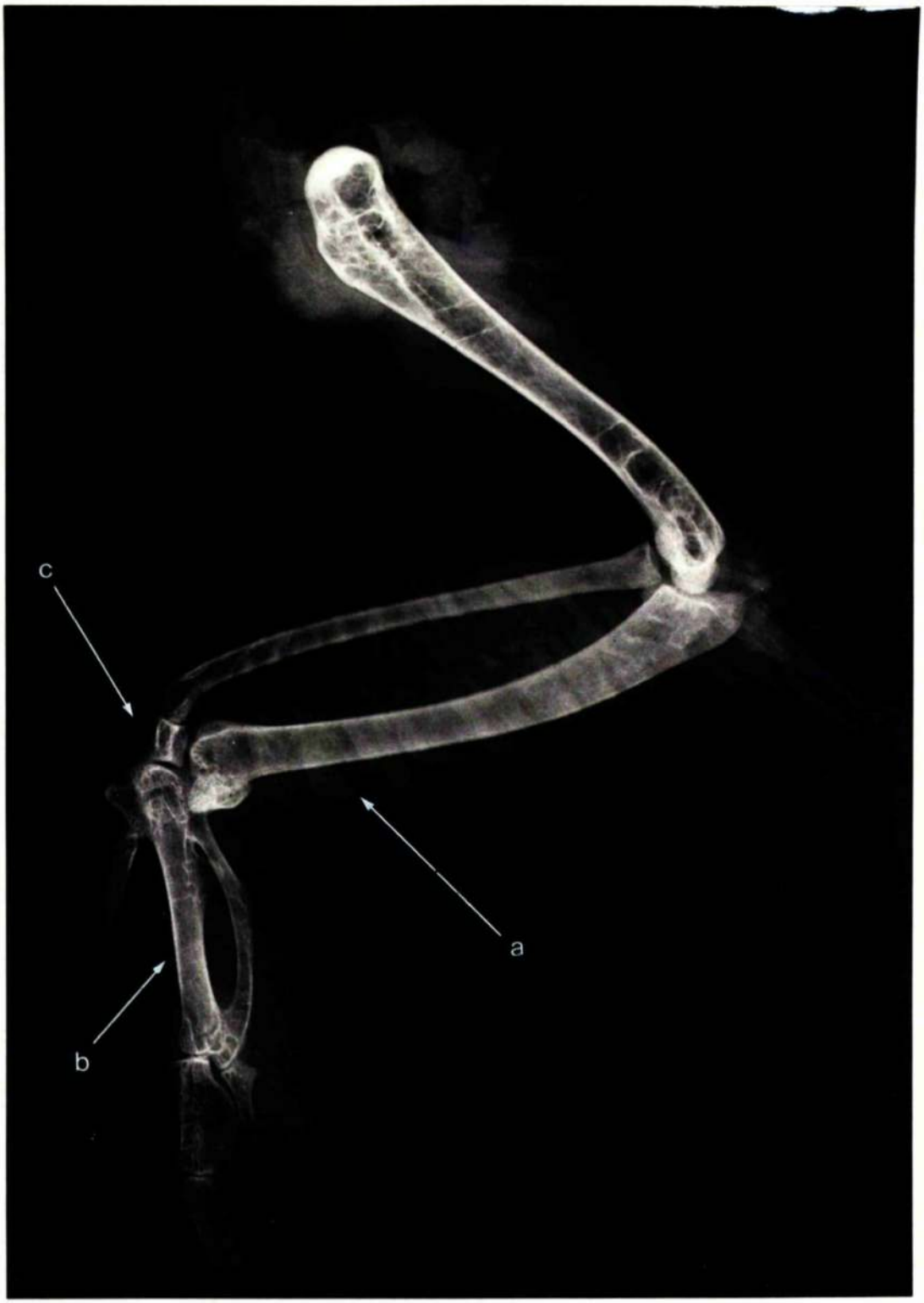


Fig. 51 Wing Adult

a. mineralised antebrachial tendon

b. mineralised tendon in dorsum of manus

c. dorsal carpal sesamoid

Radiography

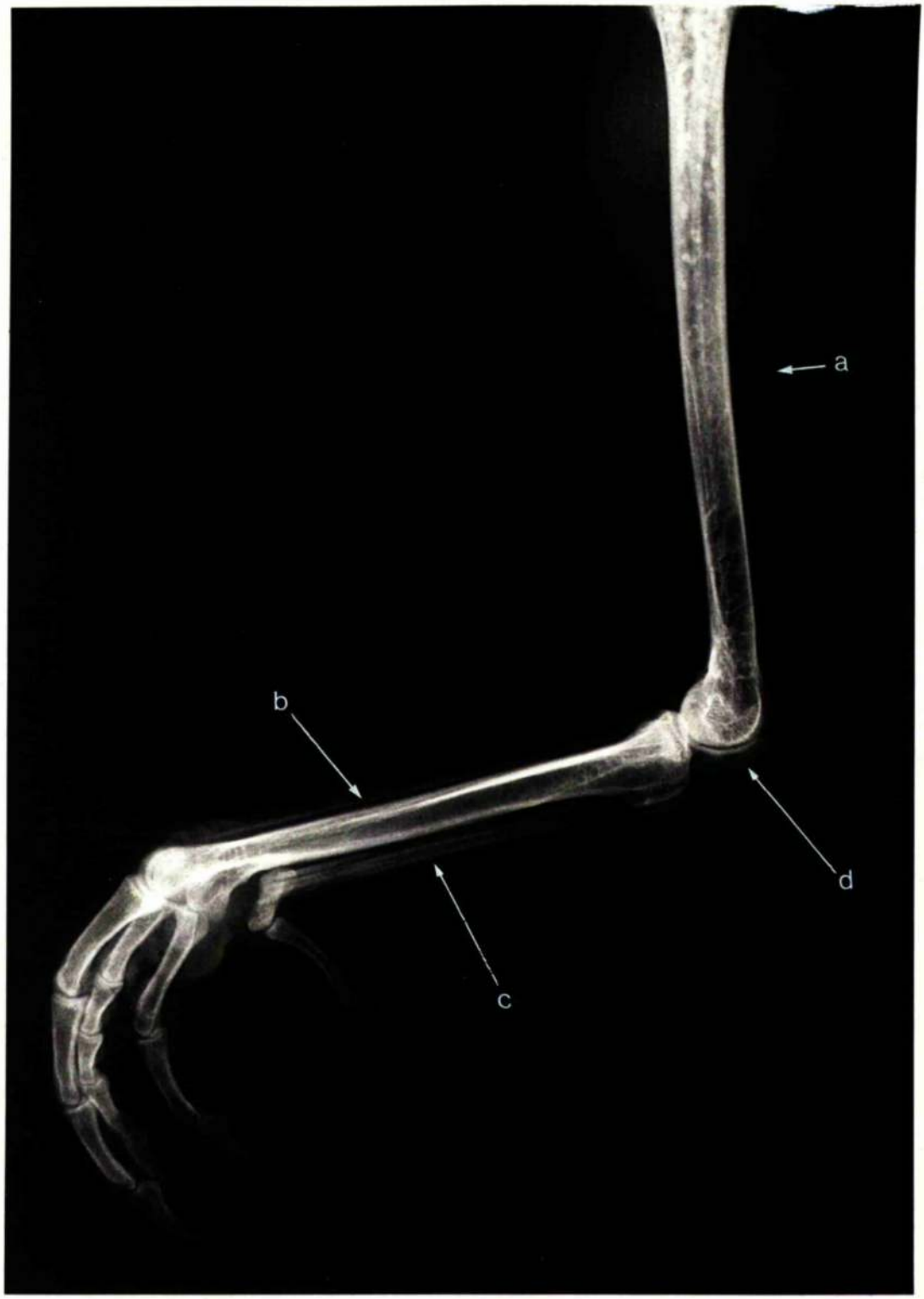


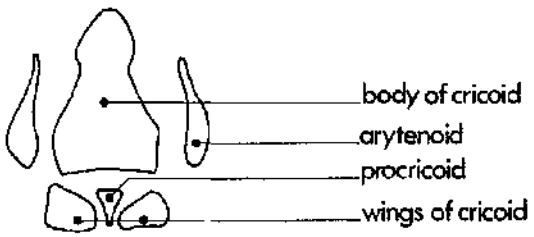
Fig. 52 Pelvic limb Adult
Lateral view Radiography

a. mineralised tendon on plantar aspect of tibiotarsus

b. mineralised tendon on dorsum of tarsometatarsus

c. 5 mineralised tendons on plantar aspect of tarsometatarsus

d. tarsal sesamoid



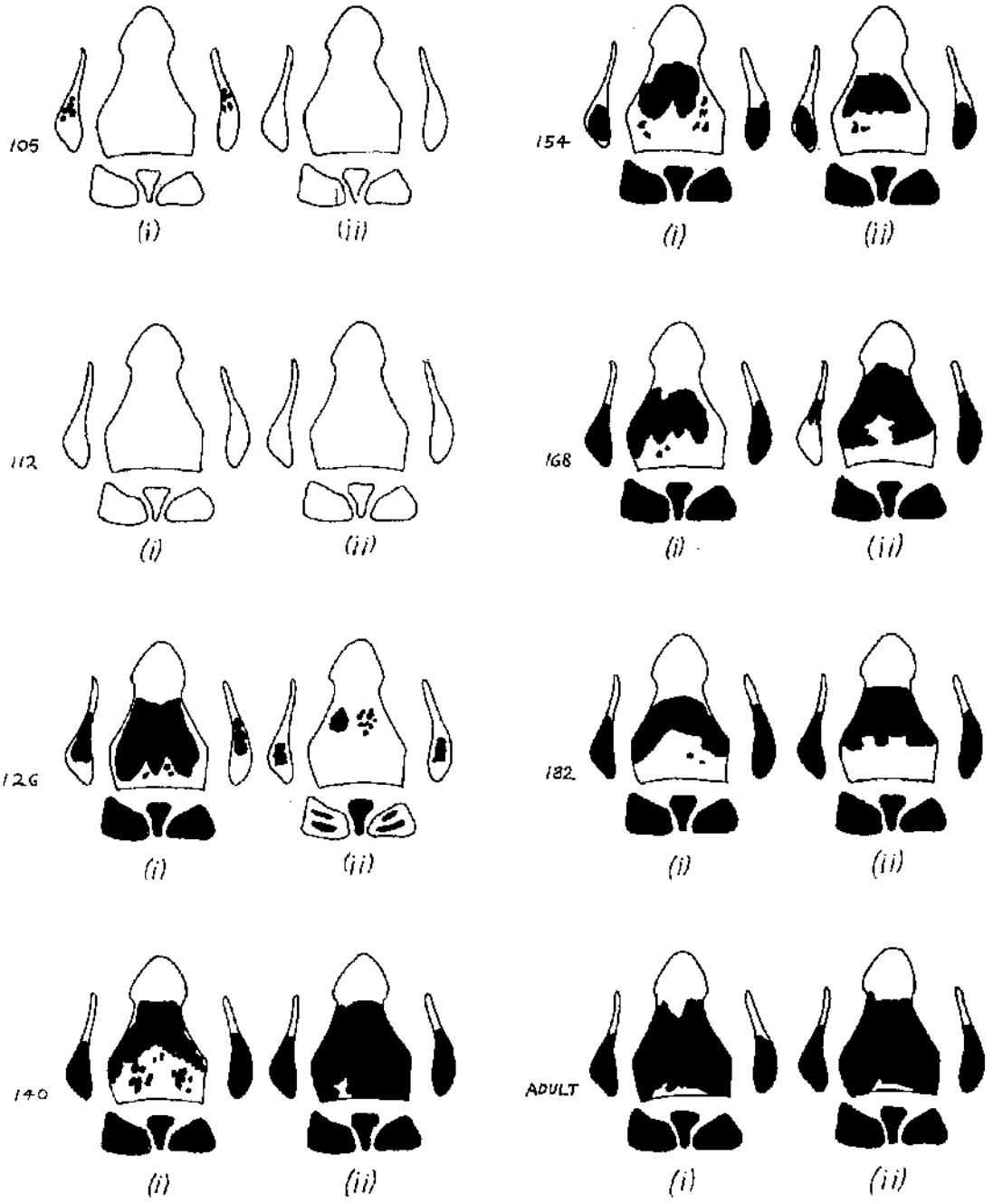


Fig. 53 Distribution of mineralisation in the laryngeal cartilages of Golden Comet birds of known ages

Fig. 54 Larynx 126 days
Ventral view
2 areas of multiple foci of mineralisation are seen in the cricoid body
Alizarin

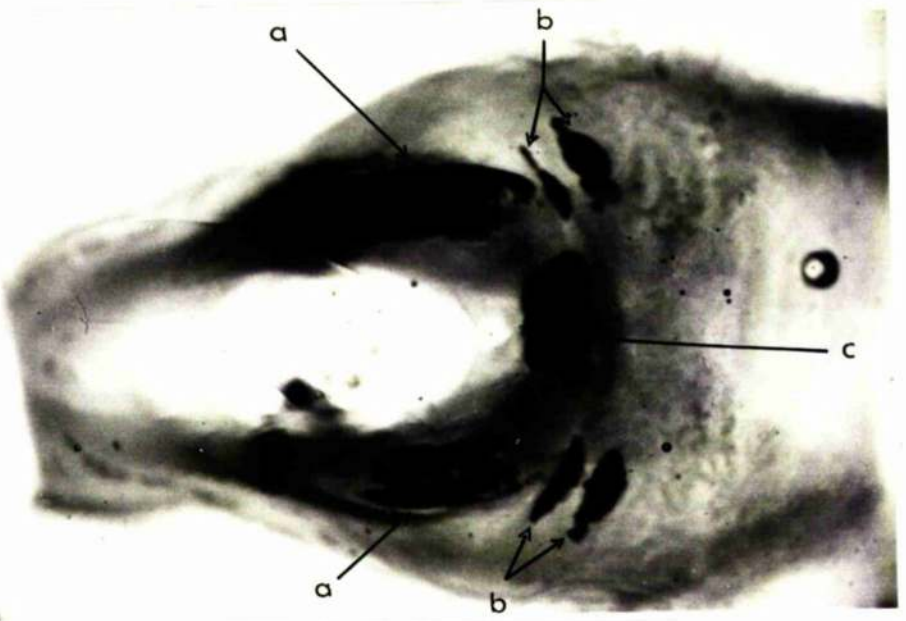


Fig. 55 Larynx 126 days

Dorsal view

a. mineralisation in the arytenoid cartilages

b. centres of mineralisation in the cricoid wings

c. mineralisation in the procricoid

Alizarin

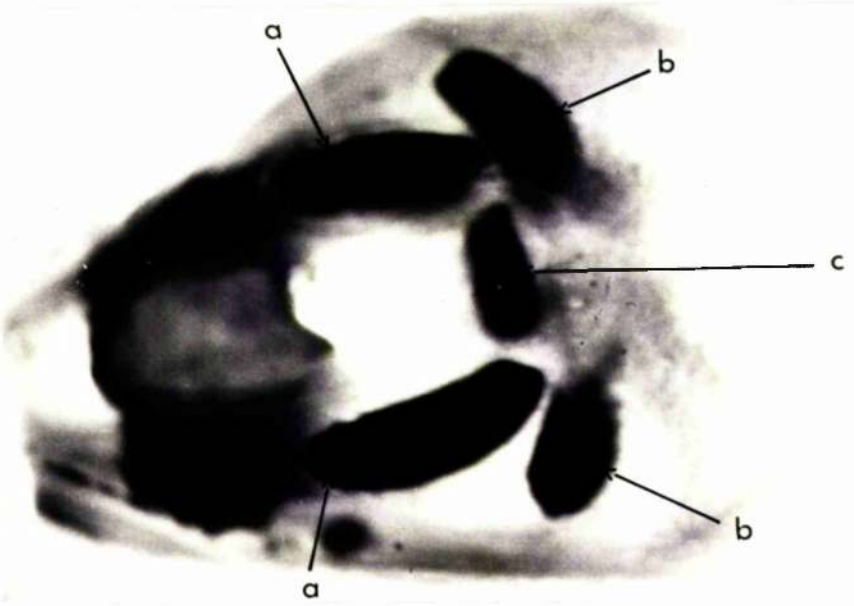


Fig. 56 Larynx 154 days

Dorsal view

a. mineralisation in base of arytenoid

b. mineralisation in cricoid wings

c. mineralisation in procricoid

Alizarin

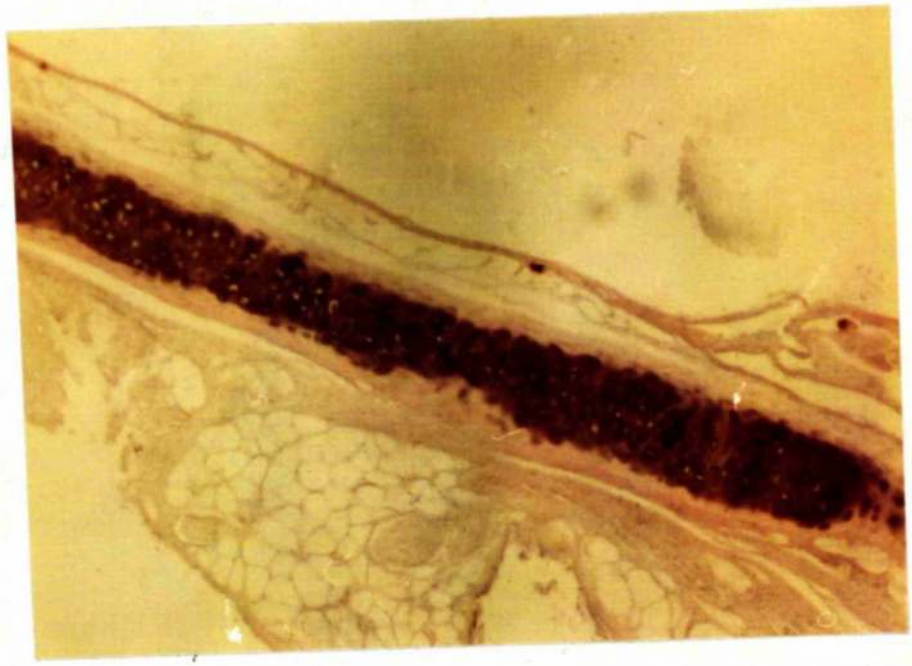


Fig. 57 Cricoid 154 days
H&E X40

Mineralised cartilage with chondrocytes arranged in distinct transverse bands. Ground substance of variable basophilic intensity. No bone deposition.

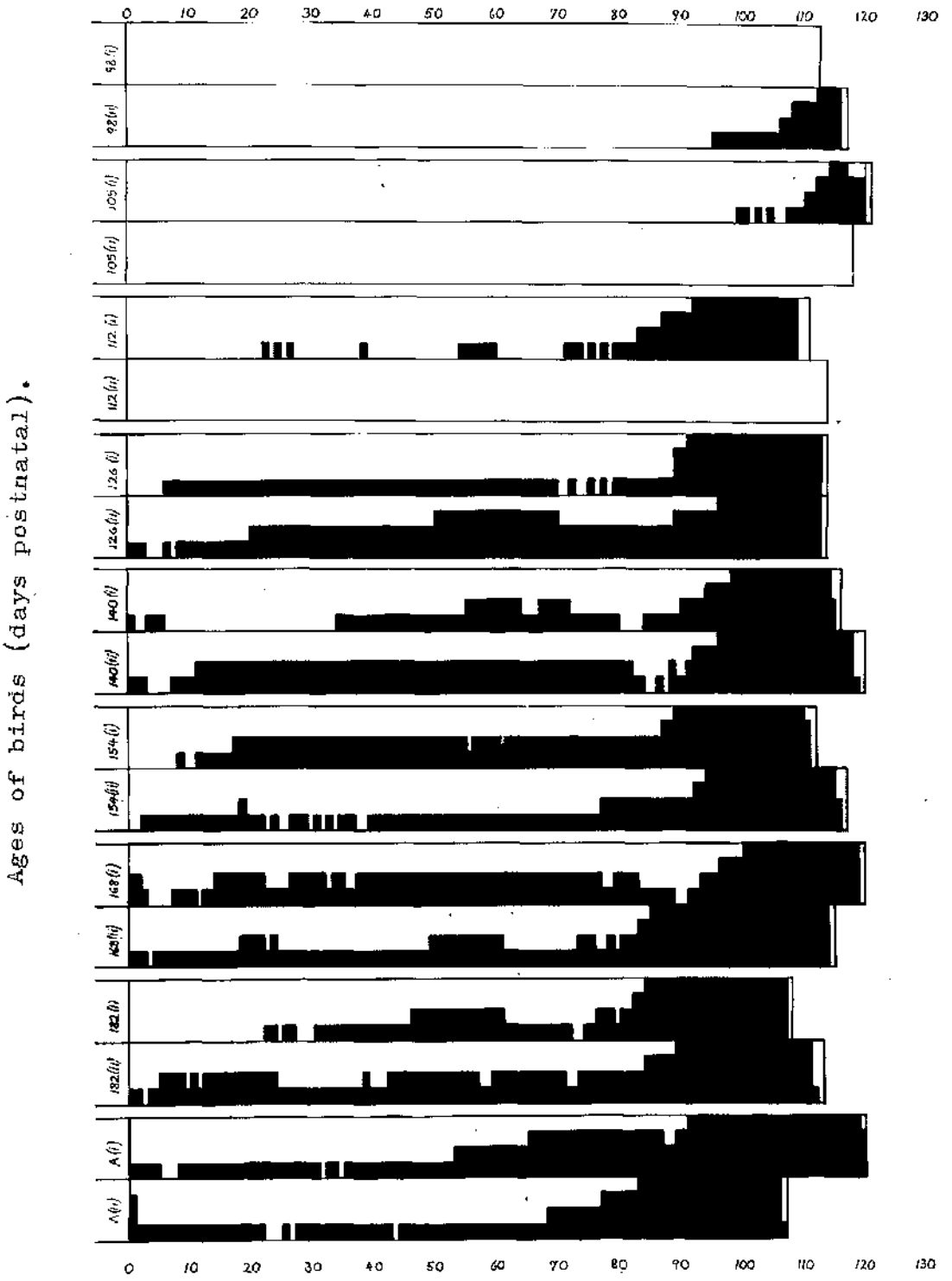
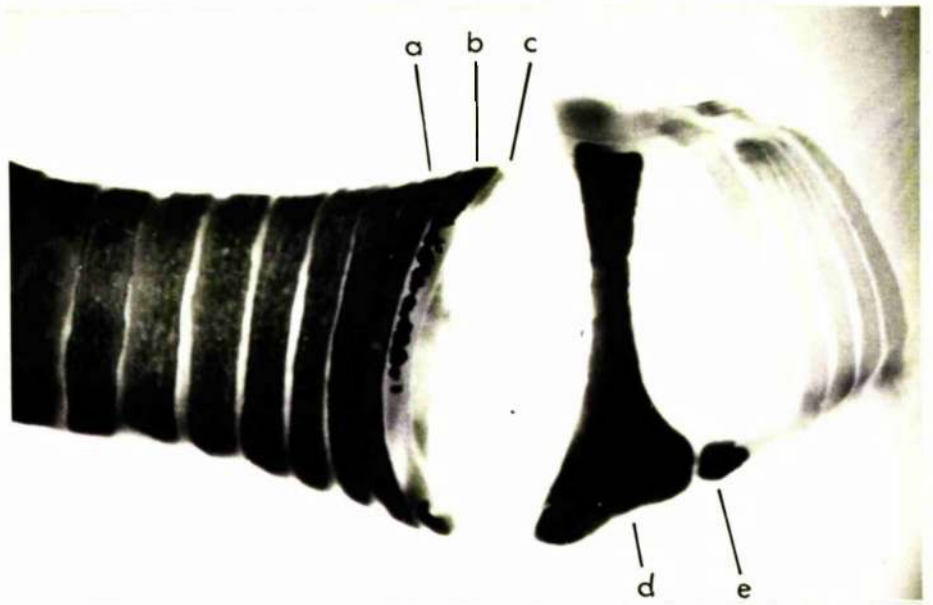
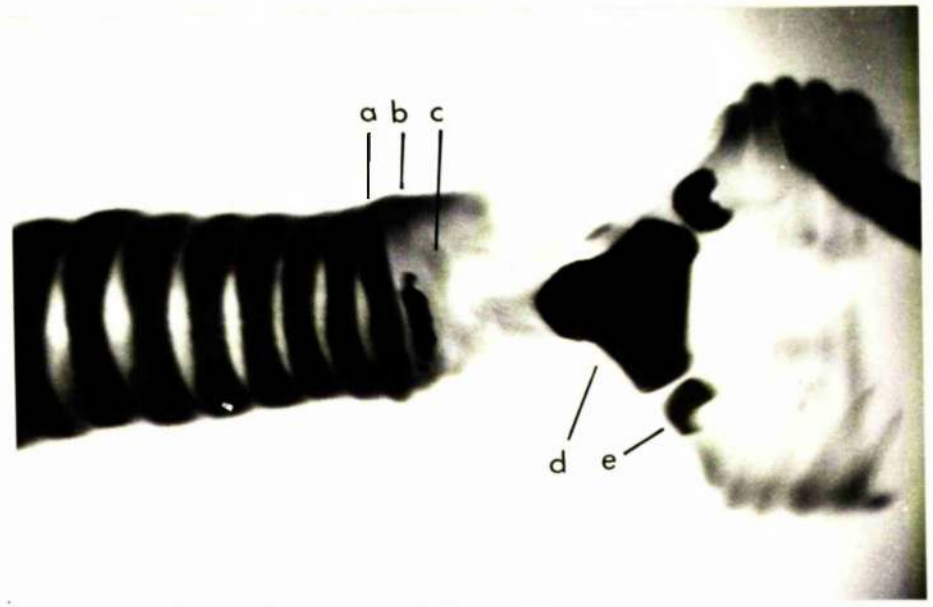


Fig. 58 Mineralisation in the tracheal rings of Golden Comet birds of known ages. Each ring is recorded as mineralised to the extent of 0, 25, 50, 75 or 100%



a



b

Fig. 59a-b Syrinx 154 days

a. left lateral view b. ventral view

a. 3rd last tracheal ring completely mineralised

b. 2nd last tracheal ring partially mineralised

c. last tracheal ring not mineralised

d. fully mineralised pessulus

e. base of 1st caudal syringeal cartilage showing mineralisation

Alizarin

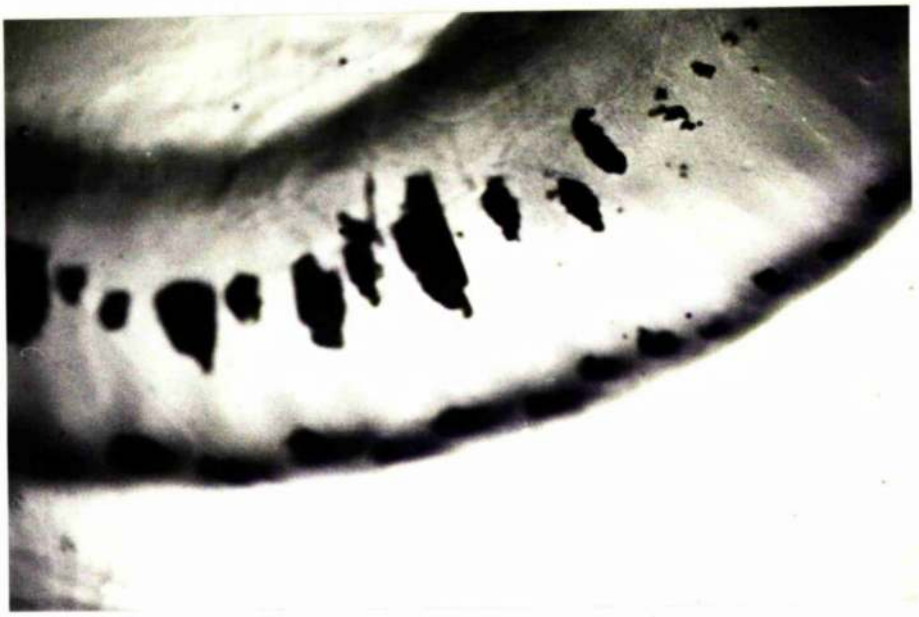


Fig. 60 Mid-trachea 154 days
Anterior view
Partial mineralisation is present in all cartilages
Alizarin

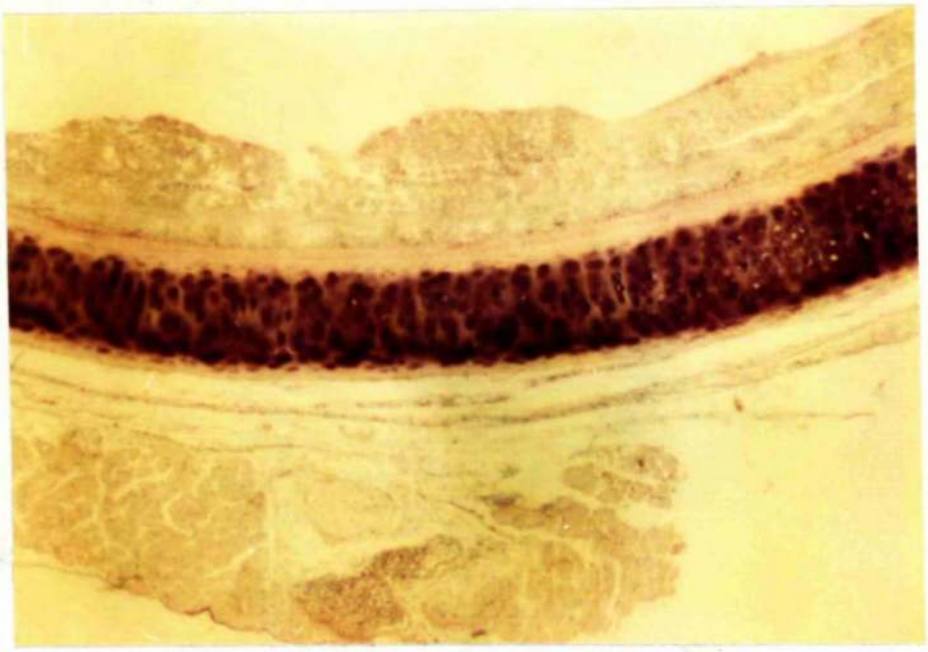


Fig. 61 Trachea 154 days
H&E X40
Complete mineralisation of cartilage. No bone deposition.

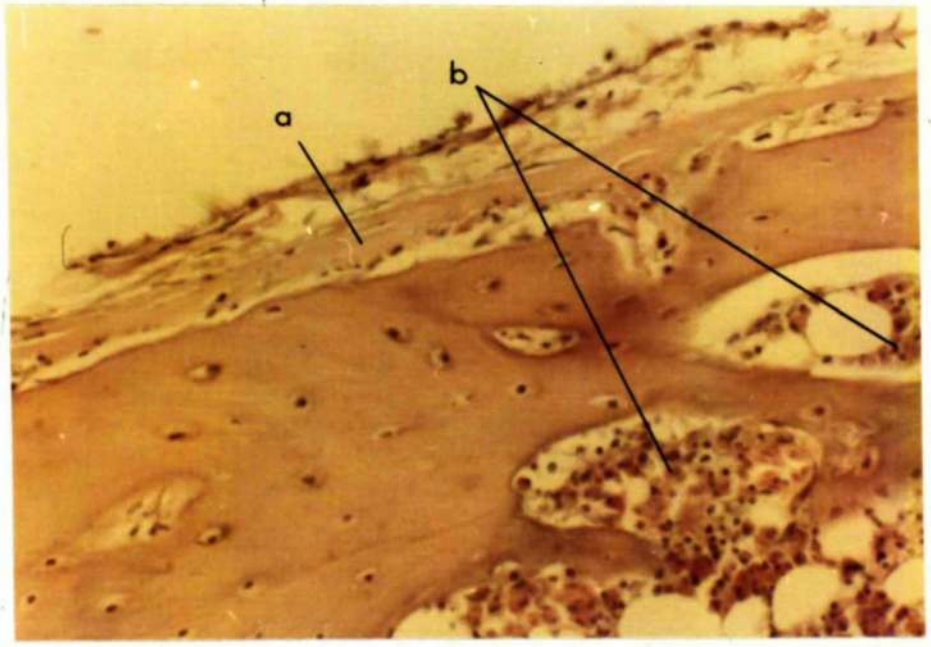
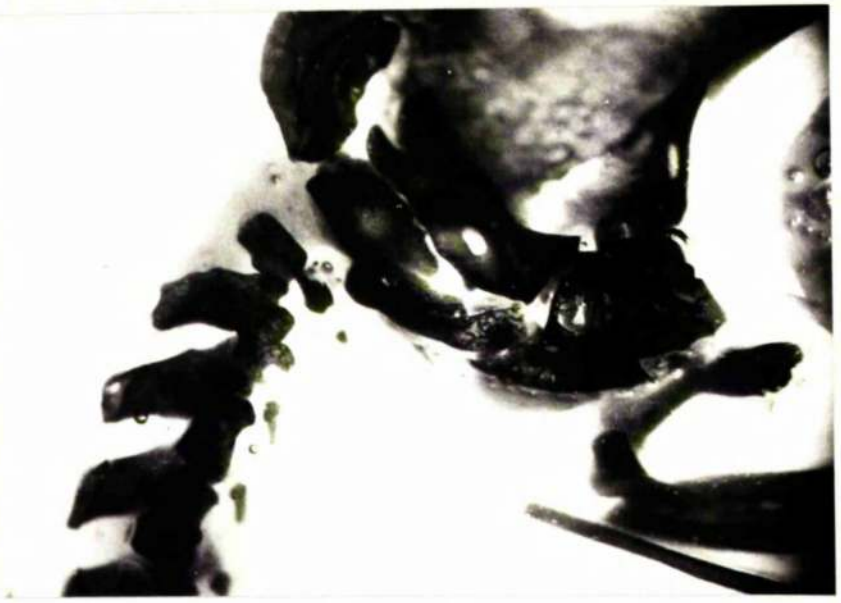


Fig. 62 Trachea 182 days
H&E X100
True ossification is present. Periosteum (a) surrounds bone spicules enclosing numerous marrow foci (b).



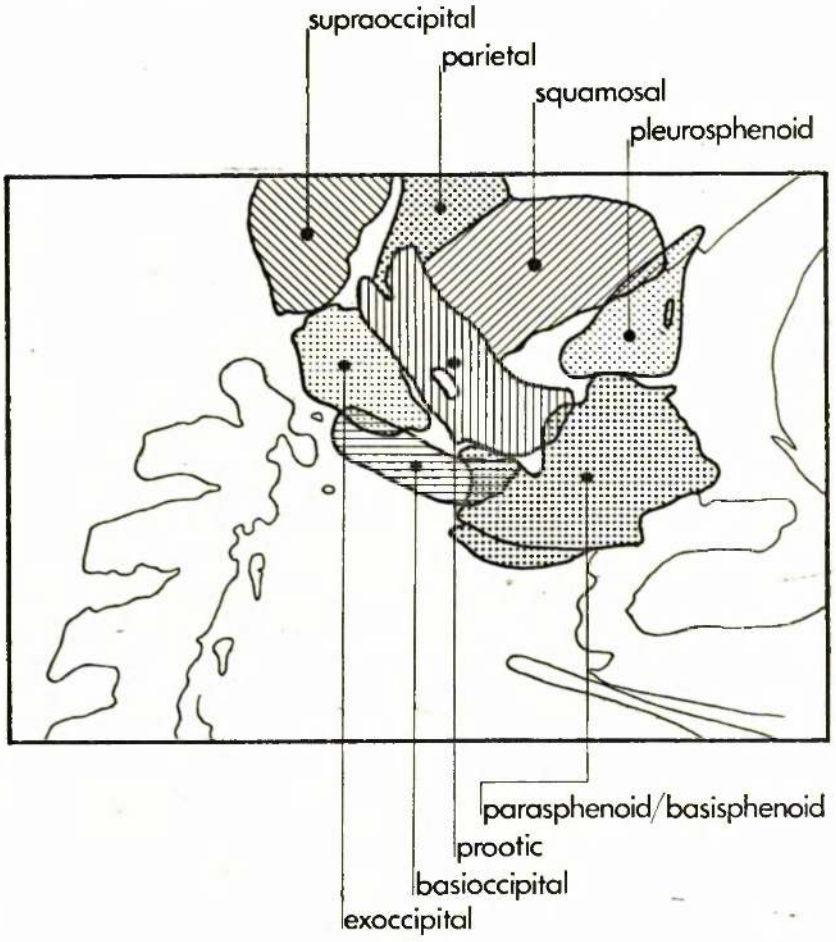


Fig. 63 Skull 0 days

Medial view of midsagittal section showing bones forming base of cranium

Alizarin

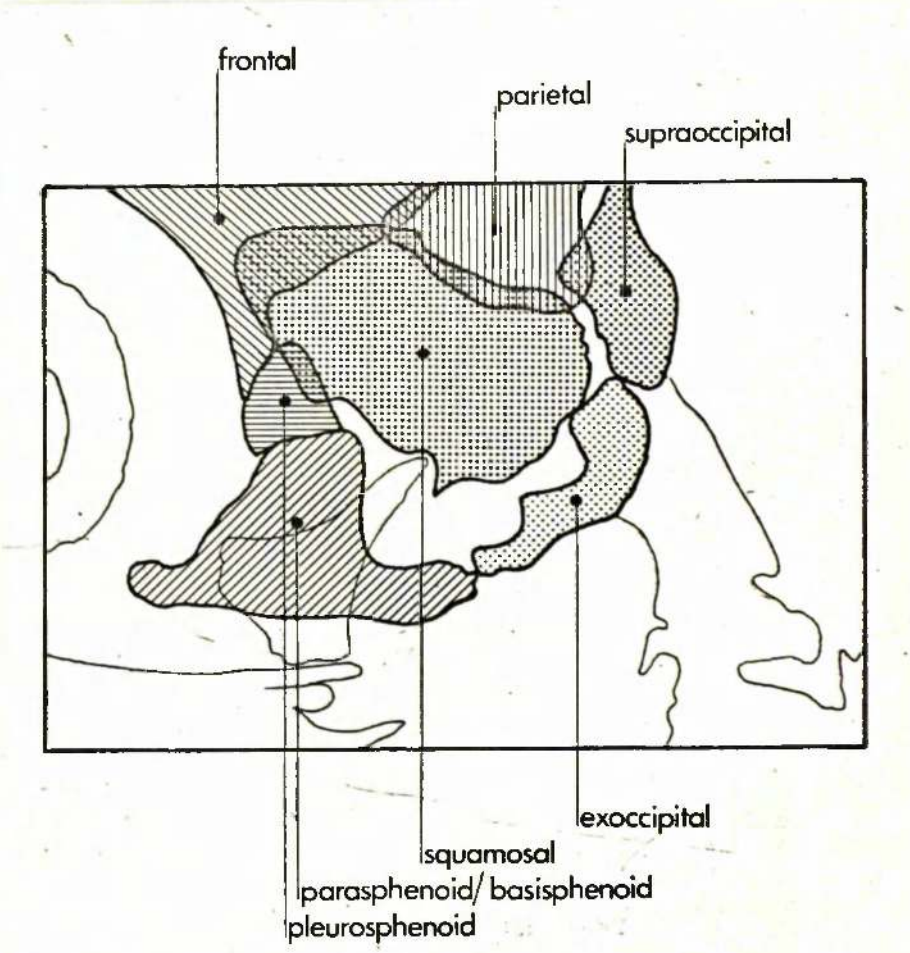


Fig. 64 Skull 0 days
Left lateral view showing squamosal and its adjacent bones
Alizarin

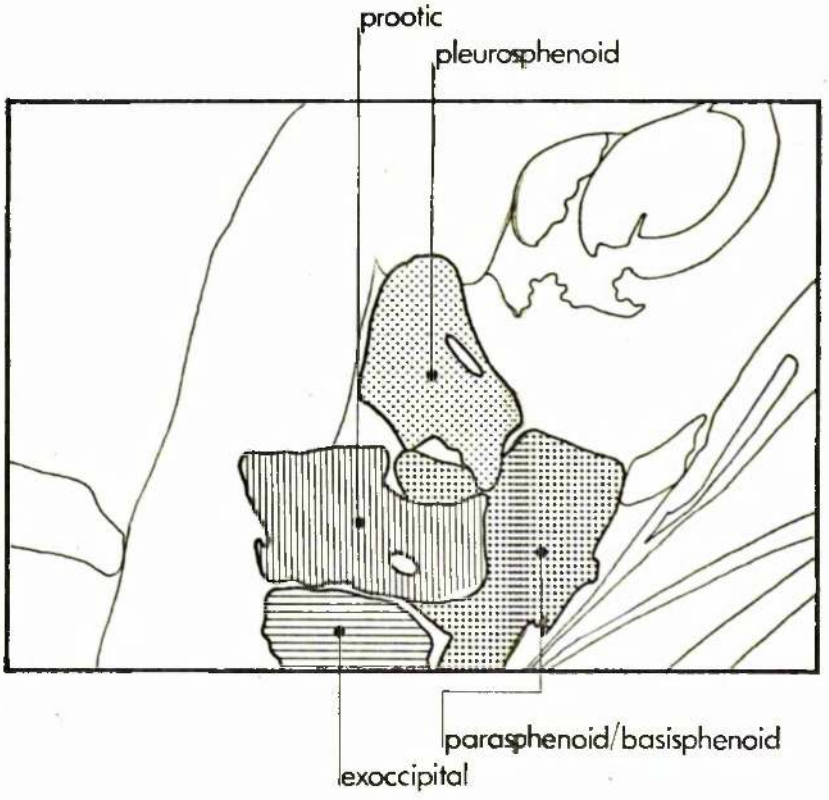


Fig. 65 Skull 0 days

Medial view of midsagittal section showing prootic and its adjacent bones

Alizarin

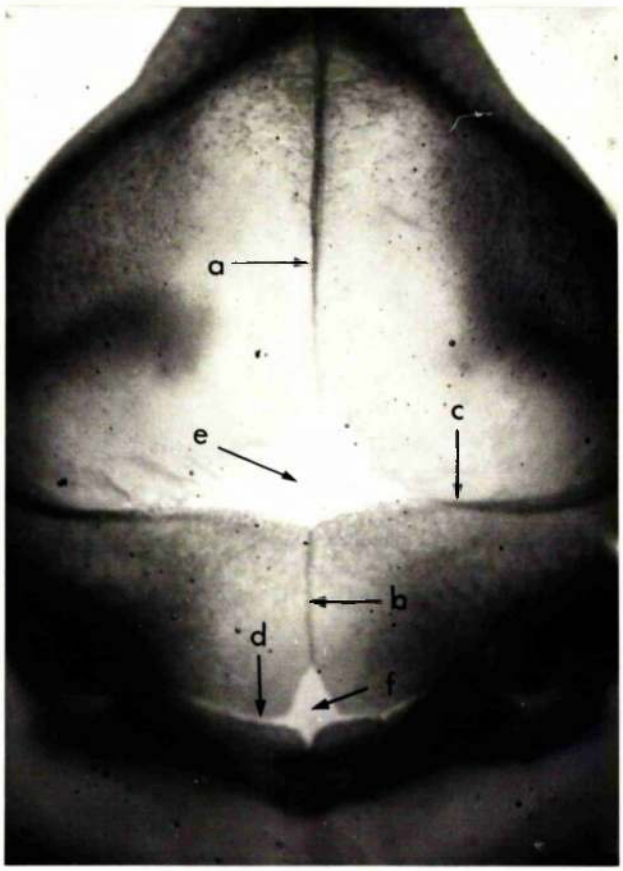


Fig. 66 Skull 0 days

Dorsal view of neurocranium

a. interfrontal suture

b. interparietal suture

c. frontoparietal suture

d. parietosupraoccipital suture

e. anterior fontanelle

f. posterior fontanelle

Alizarin

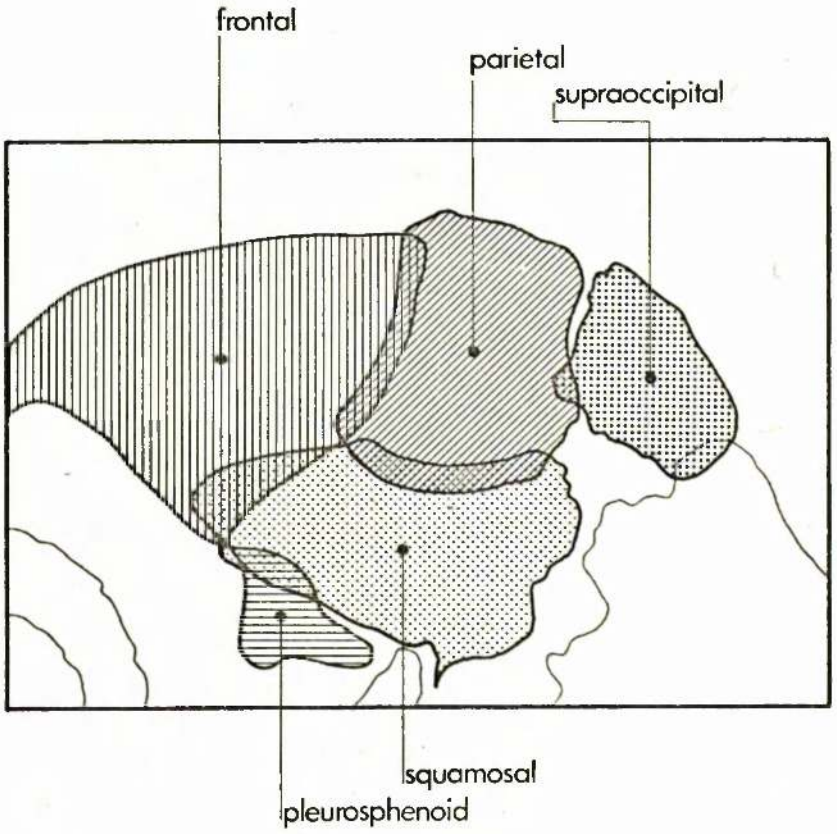
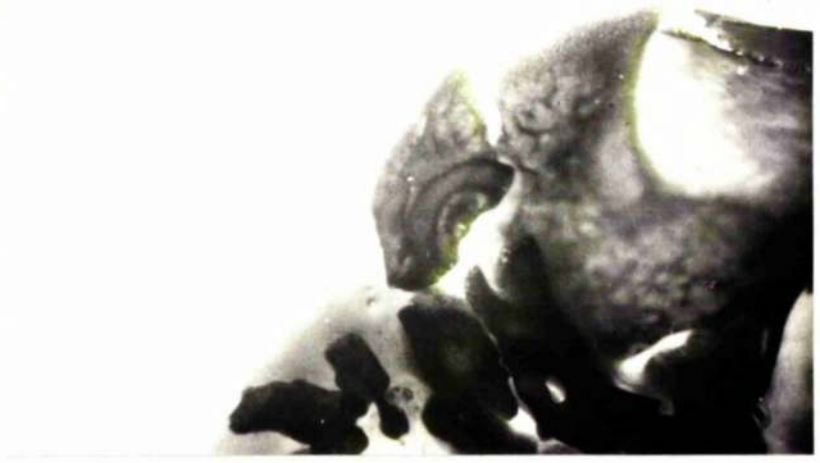


Fig. 67 Skull 0 days
Left lateral view showing parietal and
and its adjacent bones
Alizarin



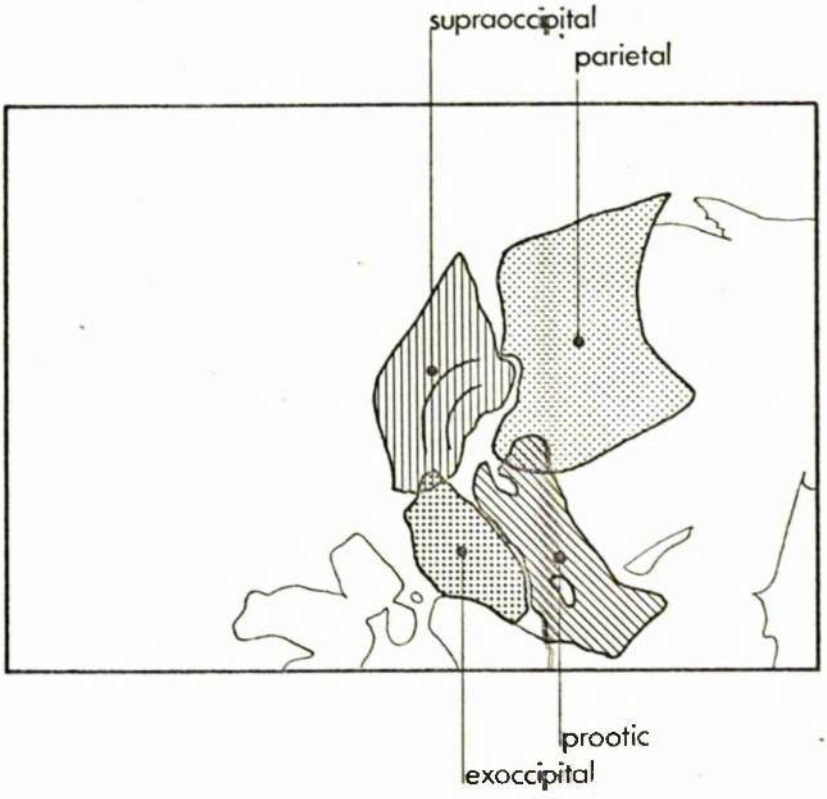
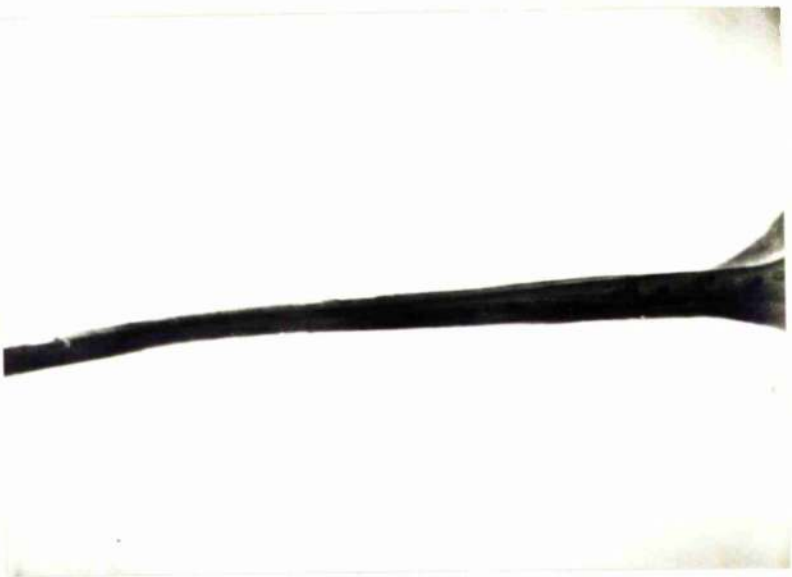


Fig. 68 Skull 0 days

Medial view of midsagittal section showing supraoccipital and its adjacent bones

Alizarin



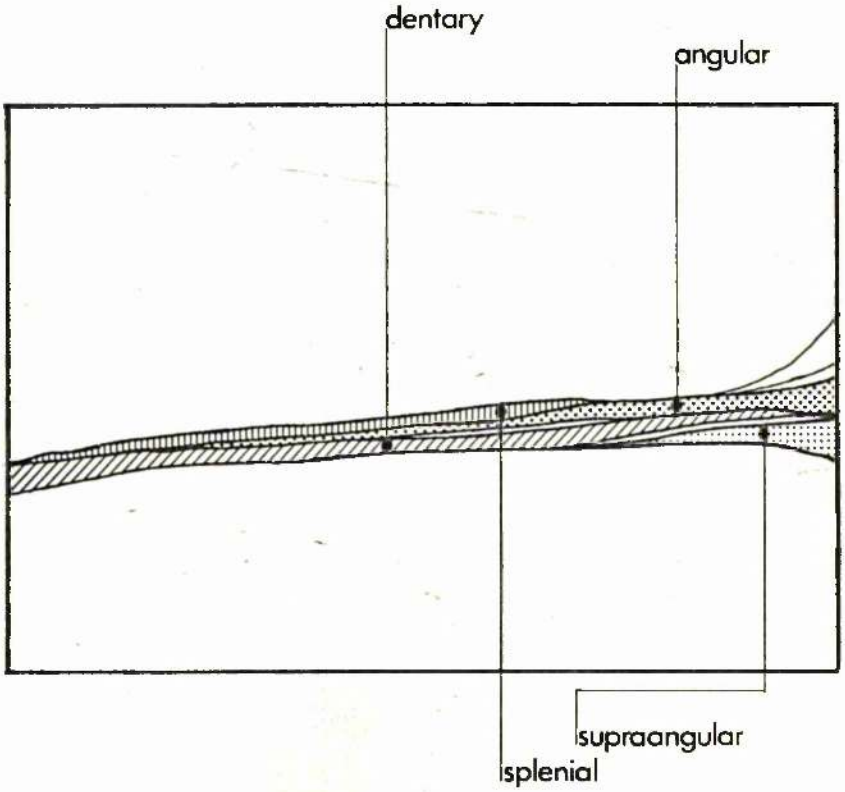
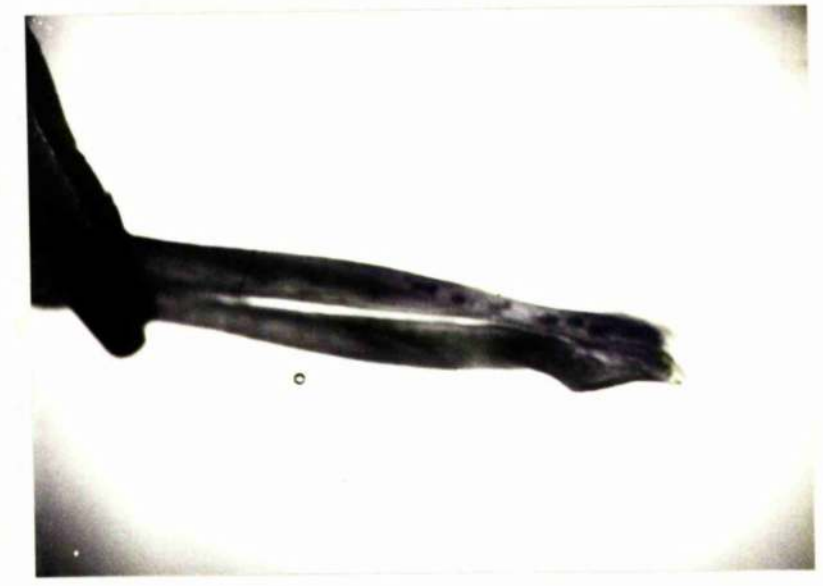


Fig. 69 Right mandible 0 days
Ventral view
Alizarin



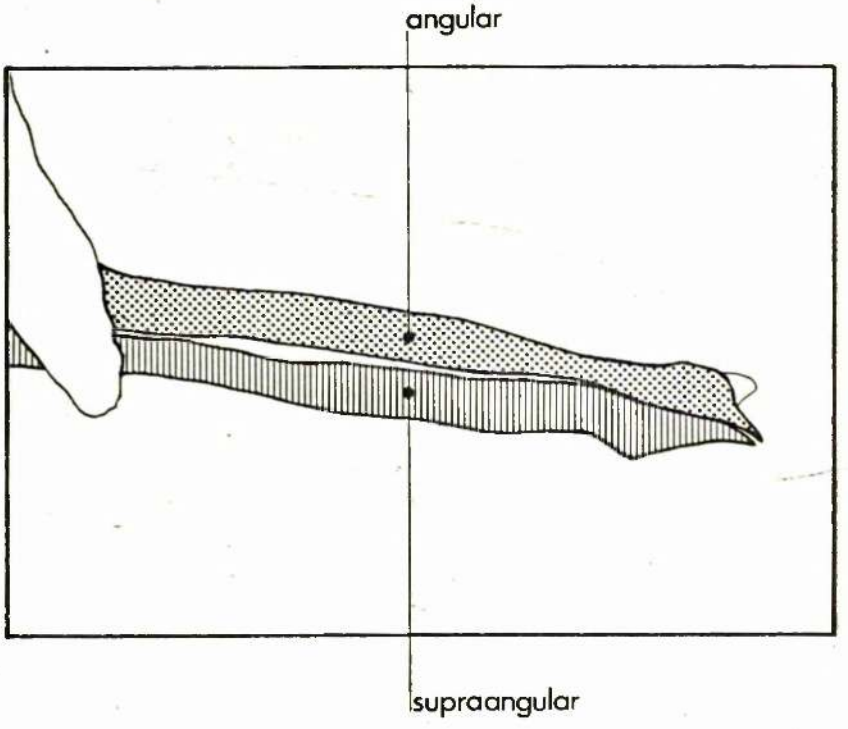


Fig. 70 Right mandible 0 days
Ventral view
Alizarin

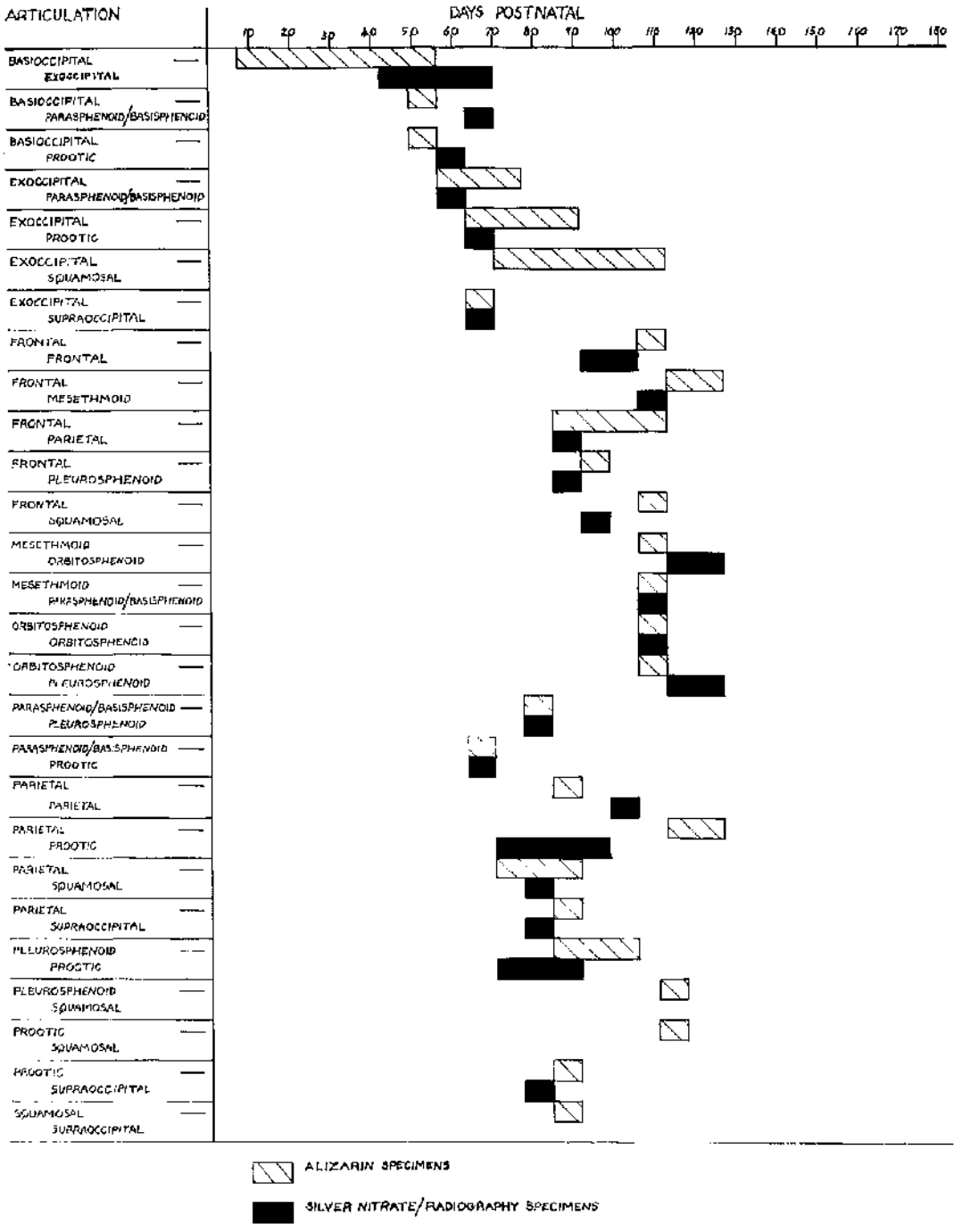


Fig. 71 Range of fusion times of skull articulations

i. neurocranium

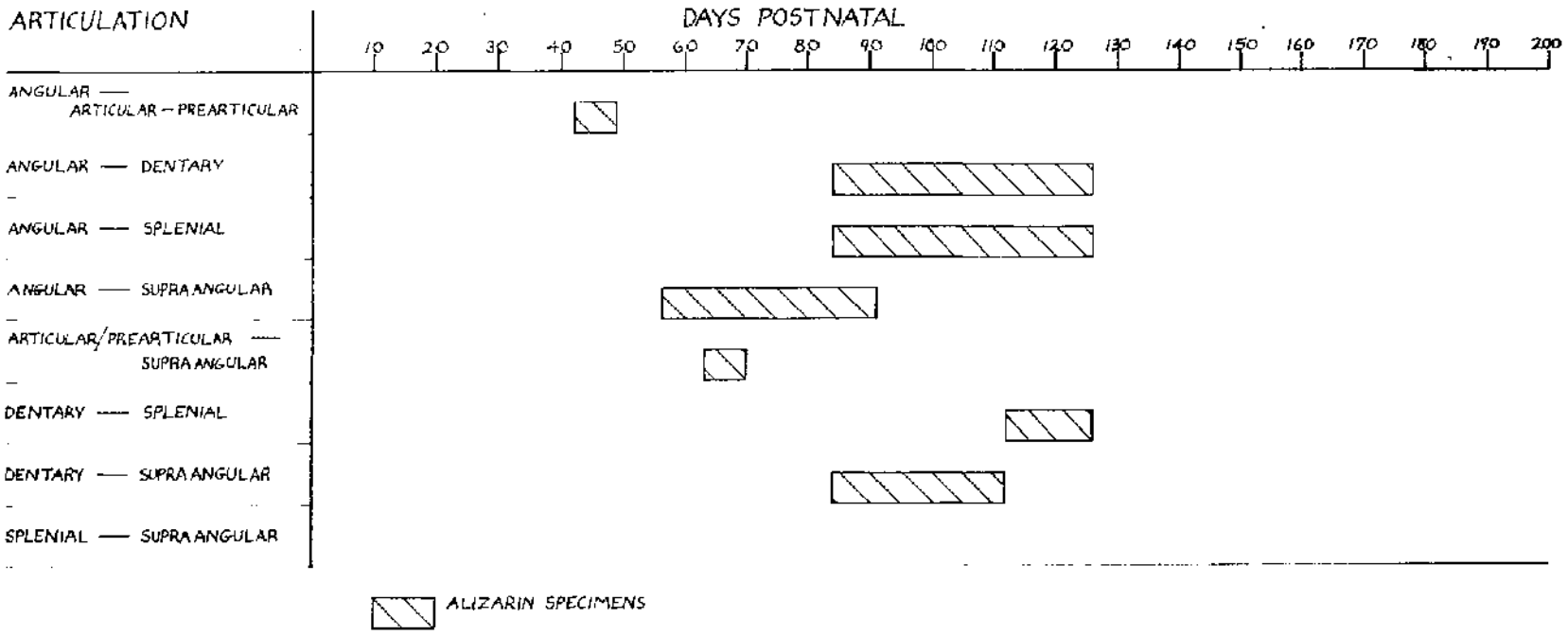


Fig. 72 Range of fusion times of skull articulations ii. mandible

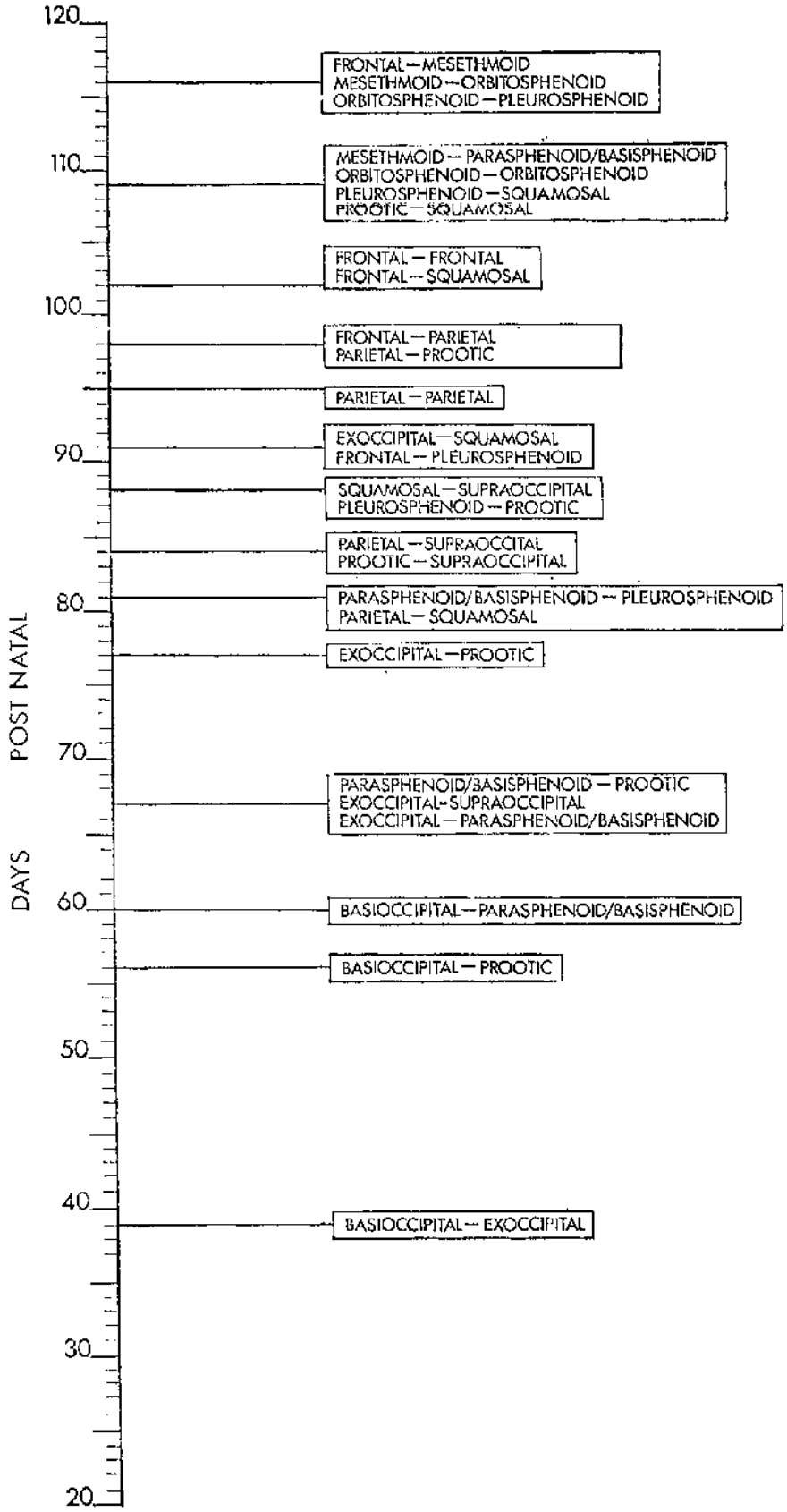


Fig. 73 Sequence of mean fusion times of skull articulations i. neurocranium

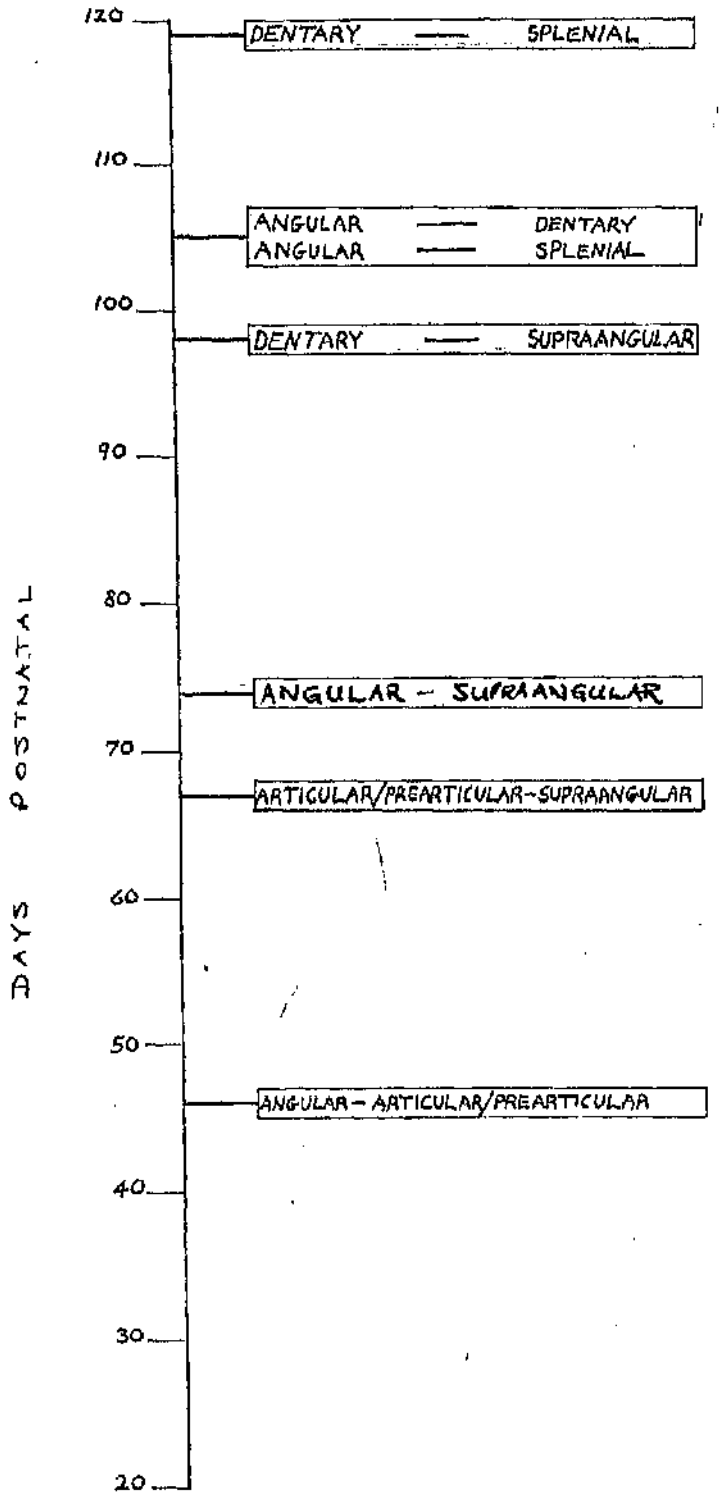
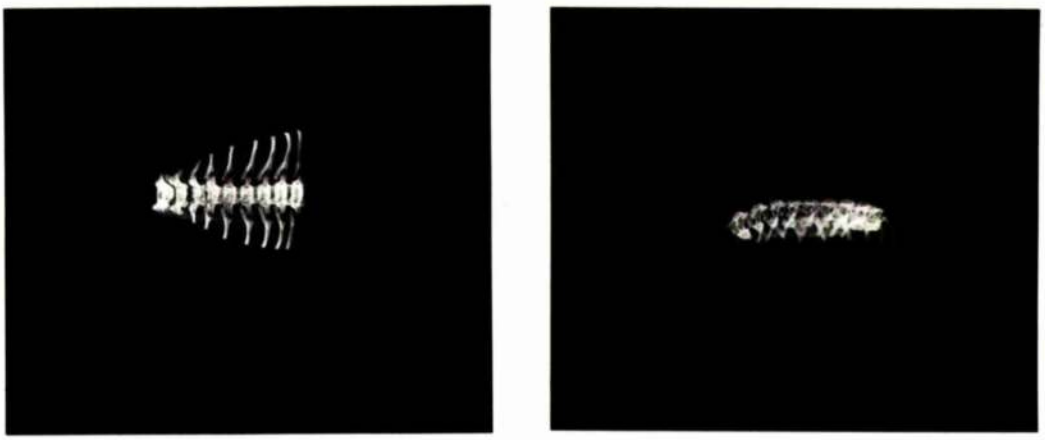
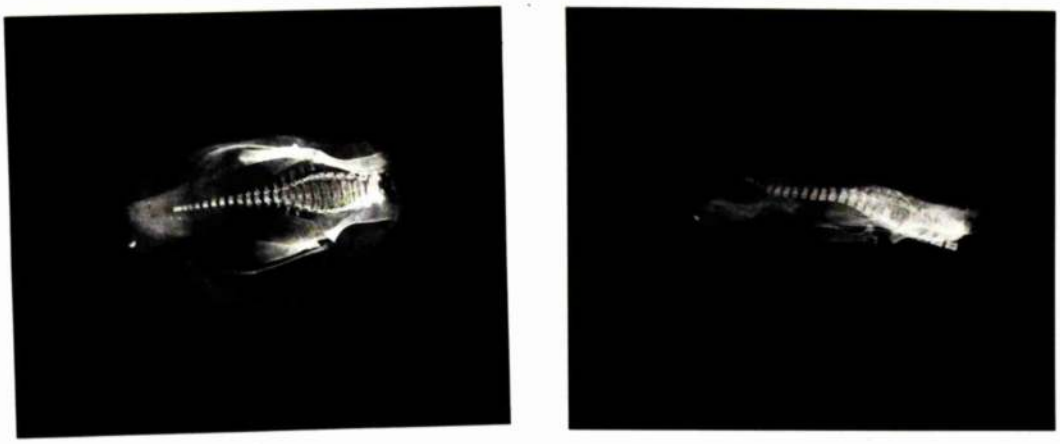


Fig. 74 Sequence of mean fusion times of skull articulations ii. mandible



a b



c d

Fig. 75 Vertebrae, ribs and os coxae 0 days
a-b Last 4 cervical and thoracics and proximal shafts of vertebral ribs
a. dorsoventral, b. lateral views
c-d Lumbosacrals and coccygeals and ossa coxarum
c. dorsoventral, d. lateral views
Silver nitrate/radiography

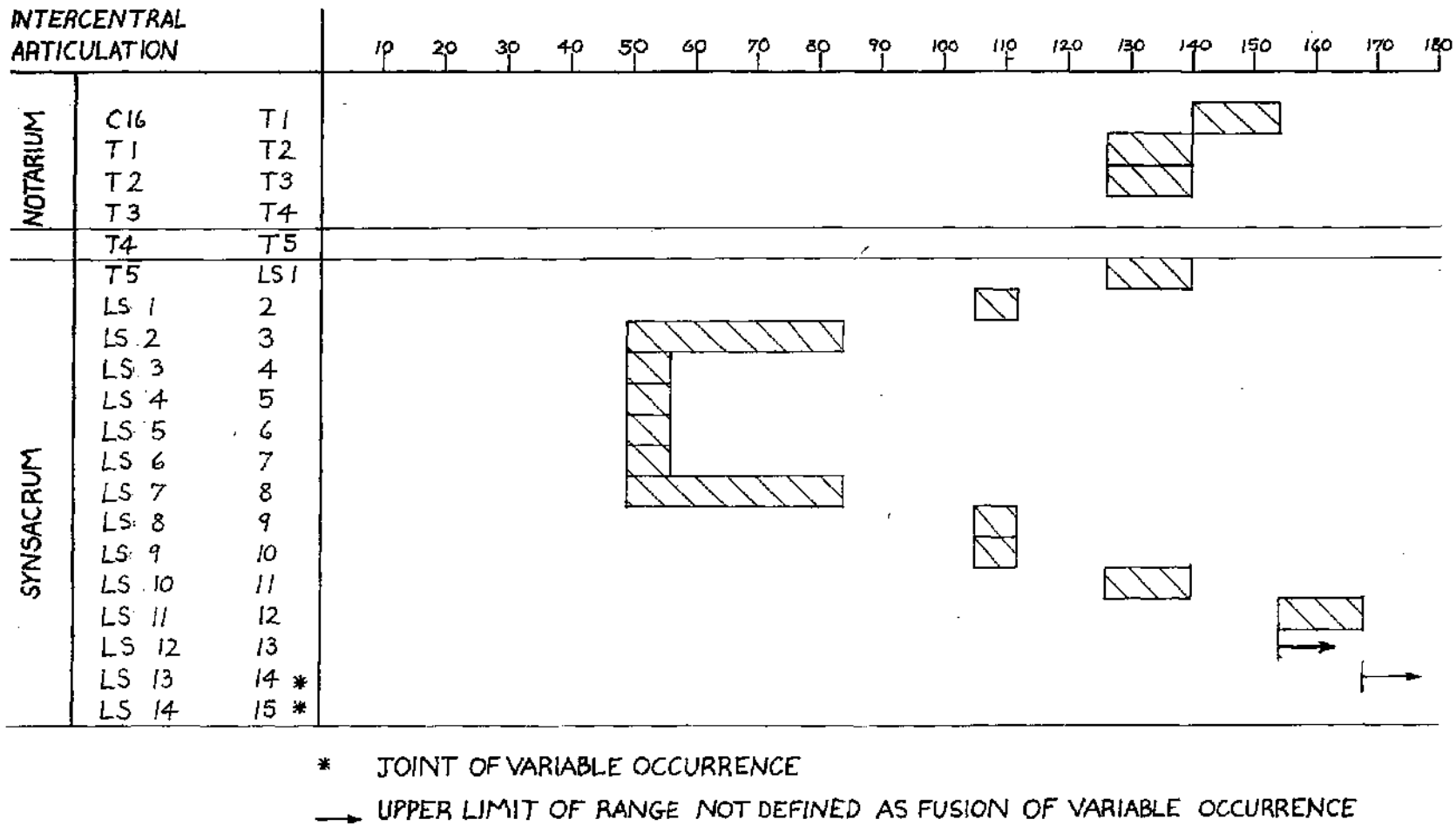


Fig. 76a Range of fusion times of intercentral articulations of thoracic and lumbosacral vertebrae

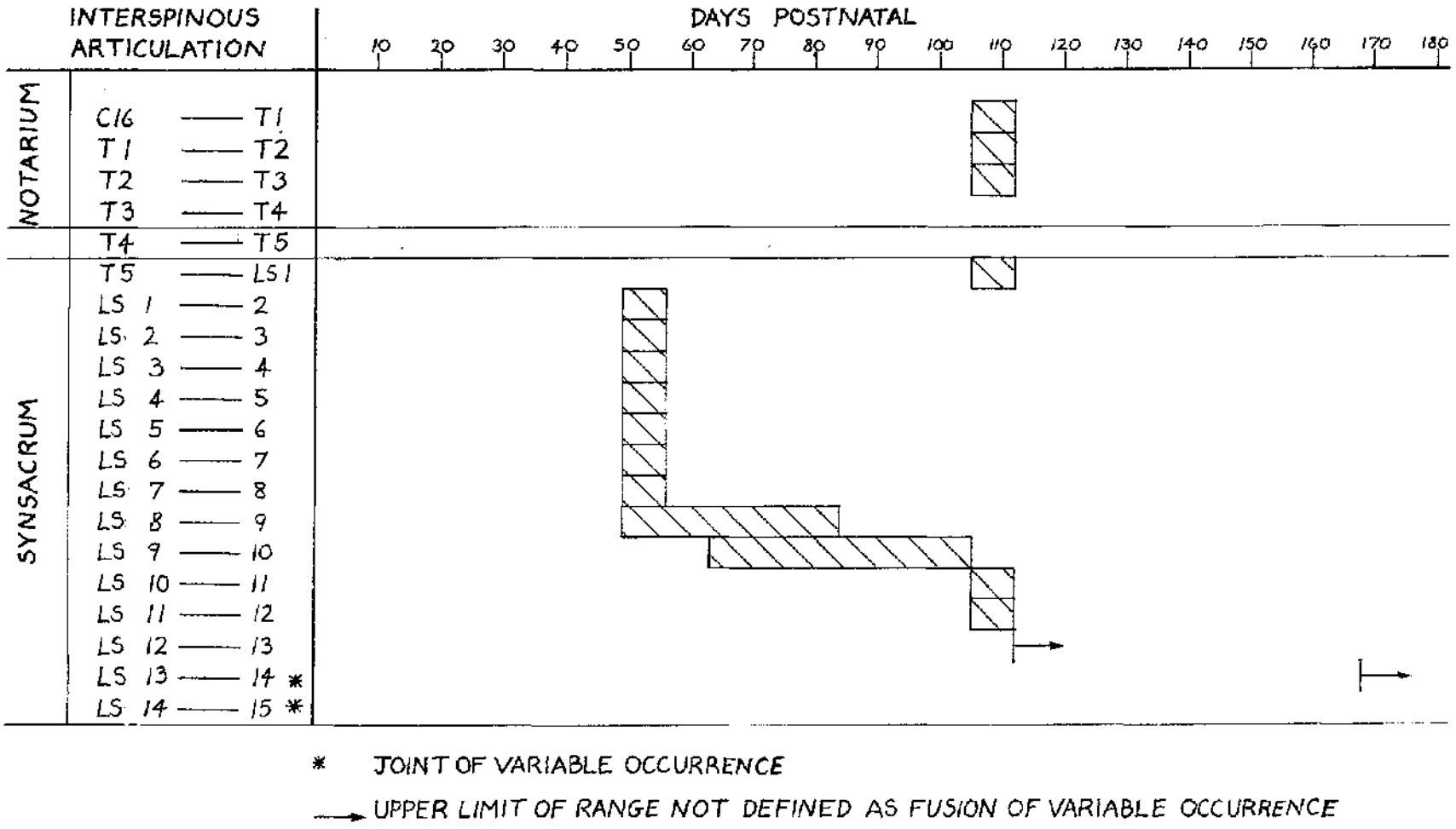


Fig. 76b Range of fusion times of interspinous articulations of thoracic and lumbosacral vertebrae

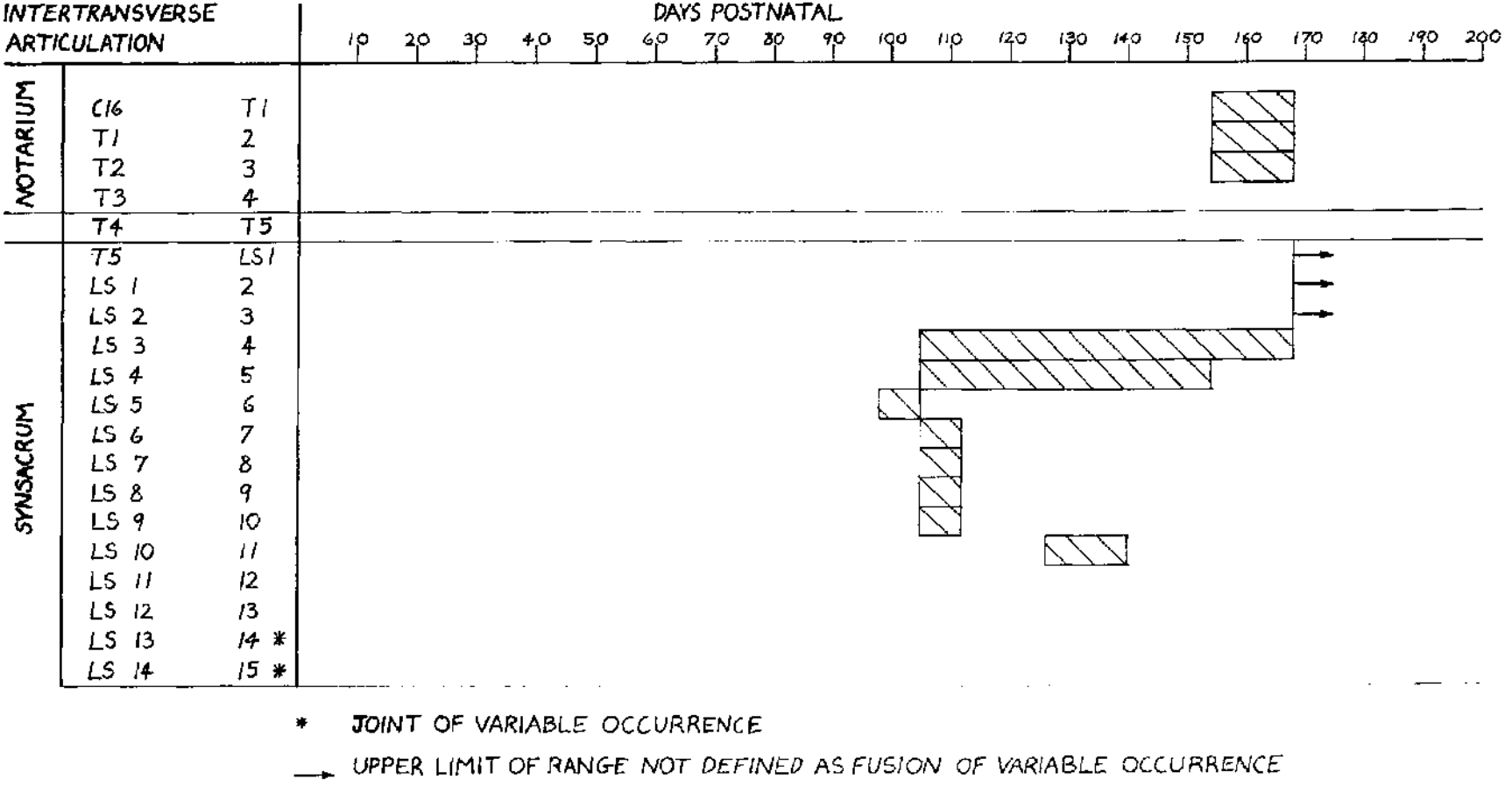


Fig. 76c Range of fusion times of intertransverse articulations of thoracic and lumbosacral vertebrae

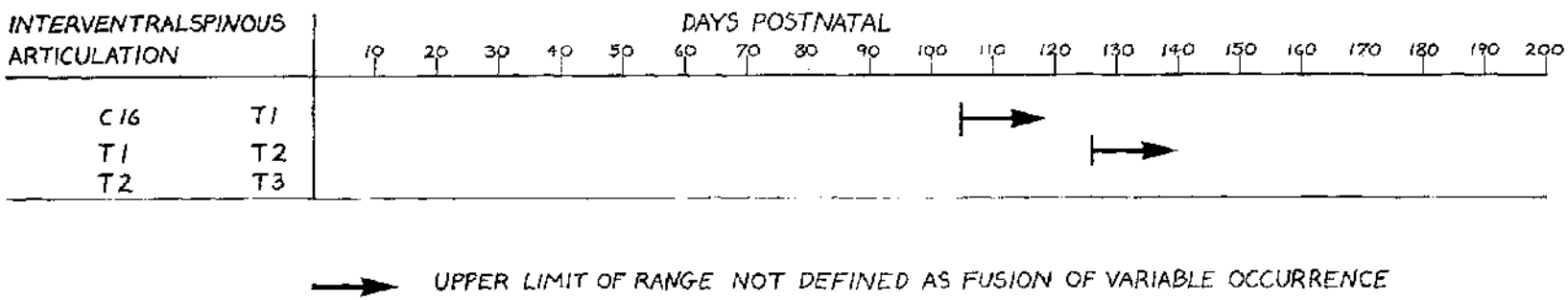


Fig. 76d Range of fusion times of articulations of ventral spines of thoracic vertebrae

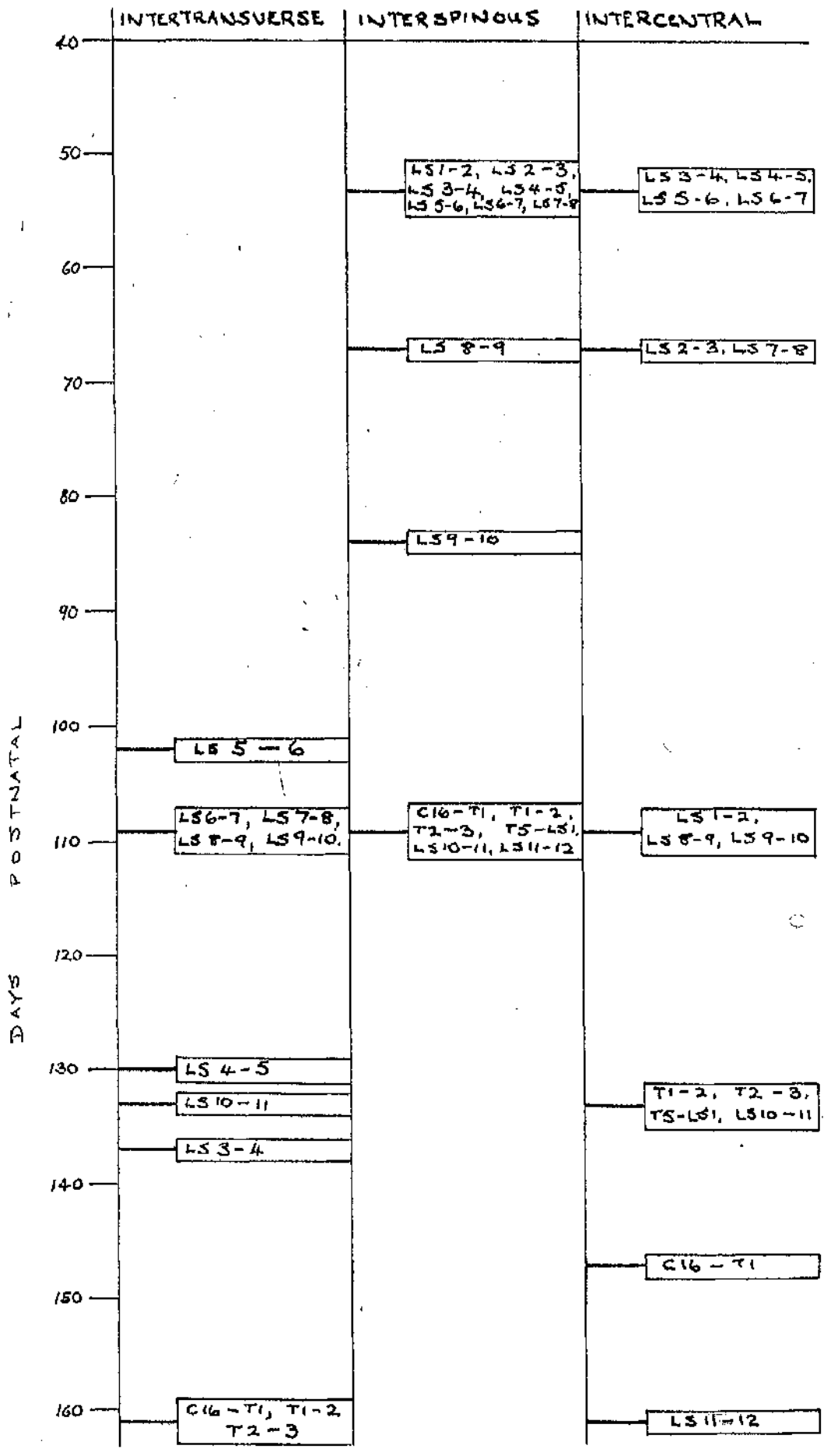


fig.77 Sequence of mean fusion times of intervertebral articulations

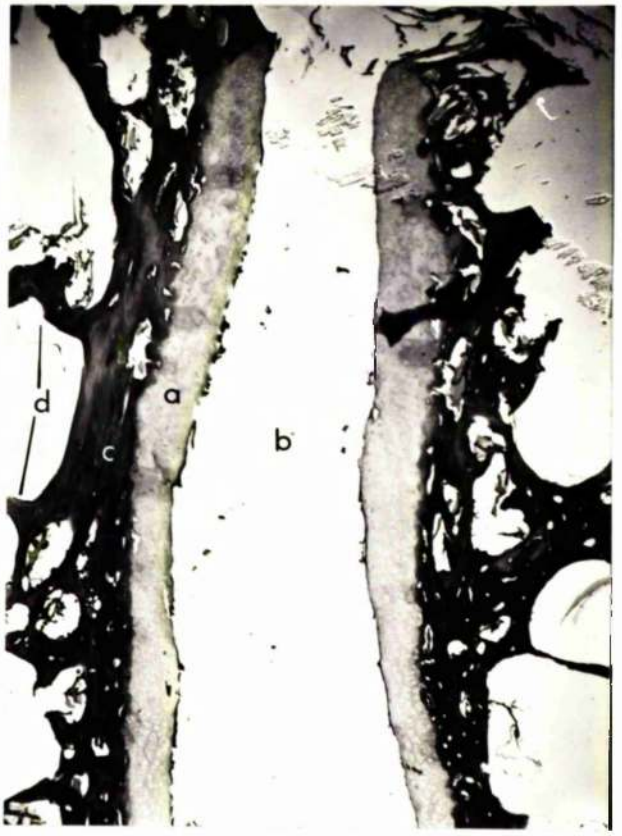


Fig. 78 Intercentral joint T4-5 Adult

a. articular cartilage

b. synovial cavity

c. compact bone

d. pneumatised trabecular bone

H&E x 25

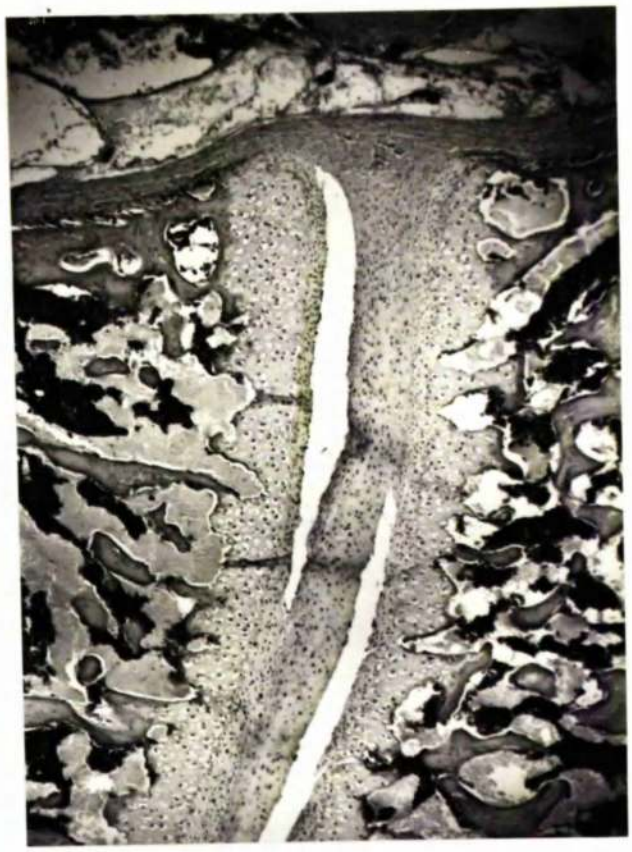


Fig. 79 **Intercentral joint T4-5** **63 days**

H&E X63

Fibrocartilage disc separating from articular cartilages.

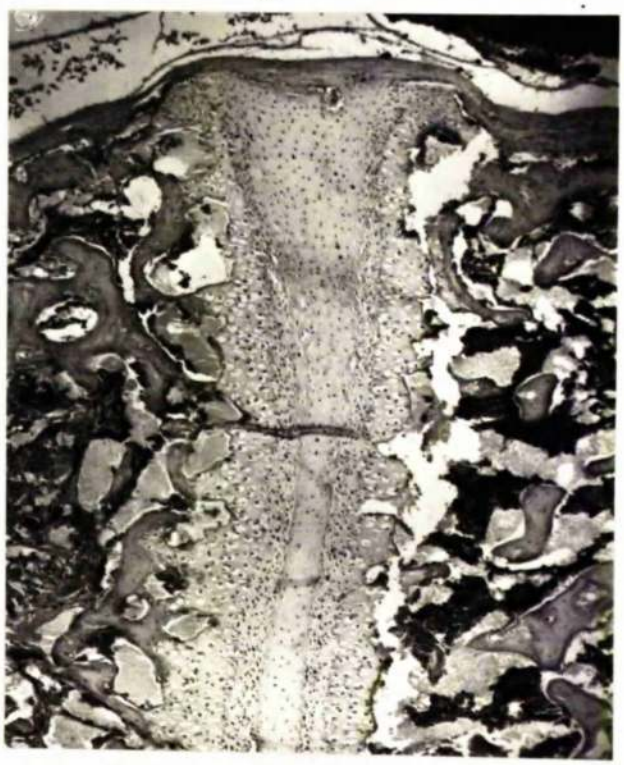


Fig. 80 **Intercentral joint T2-3** **63 days**

H&E X63

Fibrocartilage disc uniting centra.

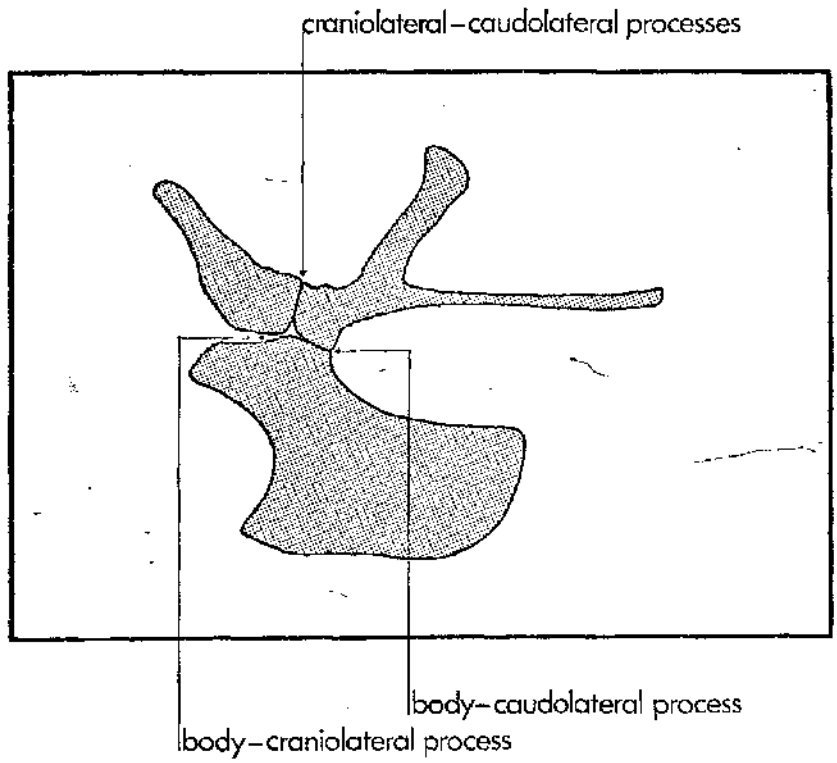


Fig. 81 Tracing of radiograph of sternum of 70 day old bird indicating future sites of fusion

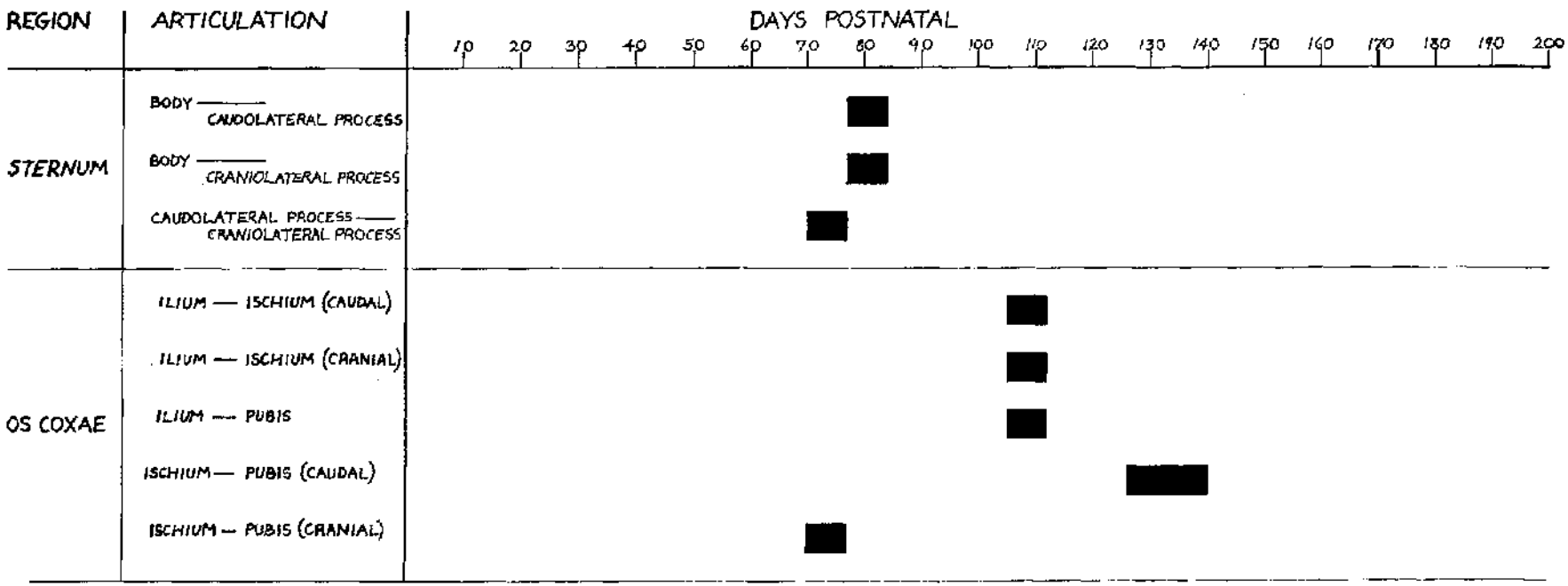


Fig. 82 Range of fusion times of articulations of sternum and os coxae

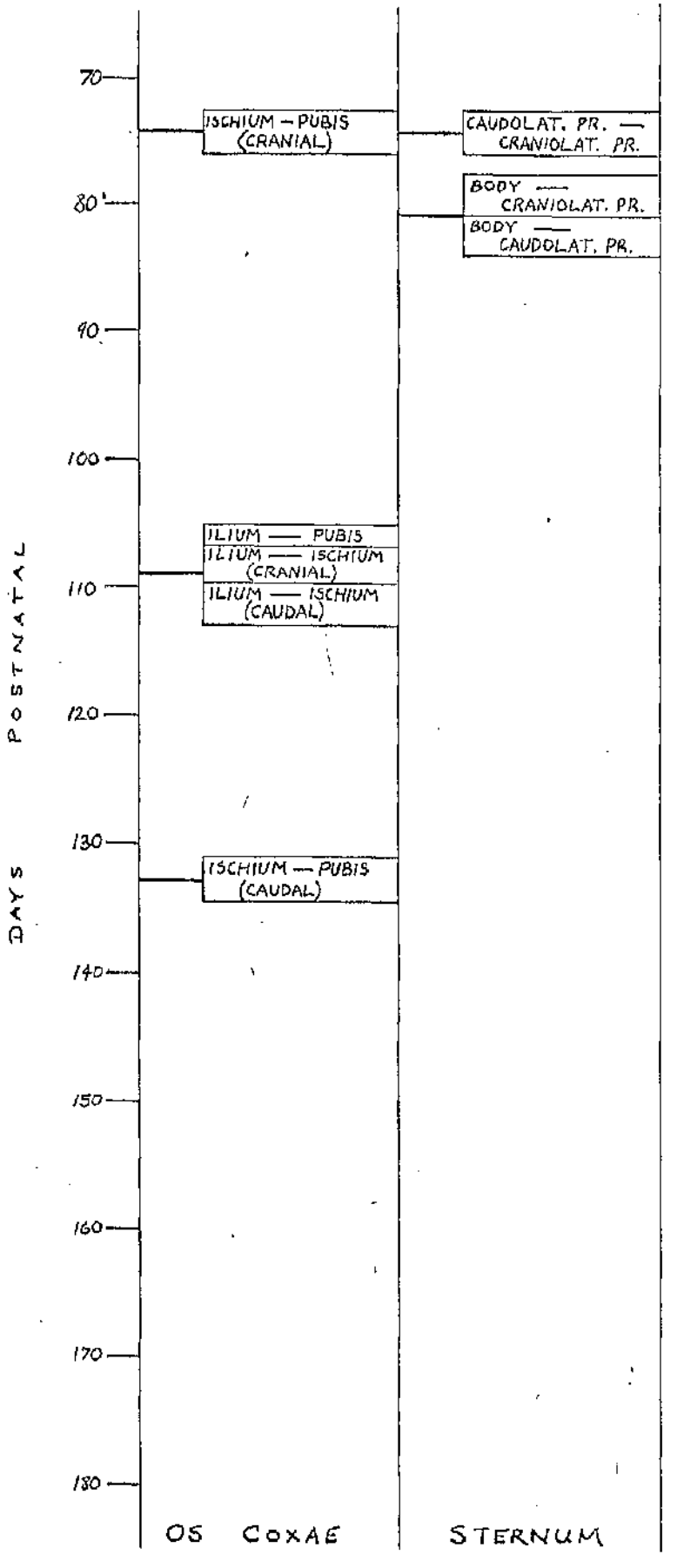


Fig. 83 Sequence of mean fusion times of articulations of sternum and os coxae

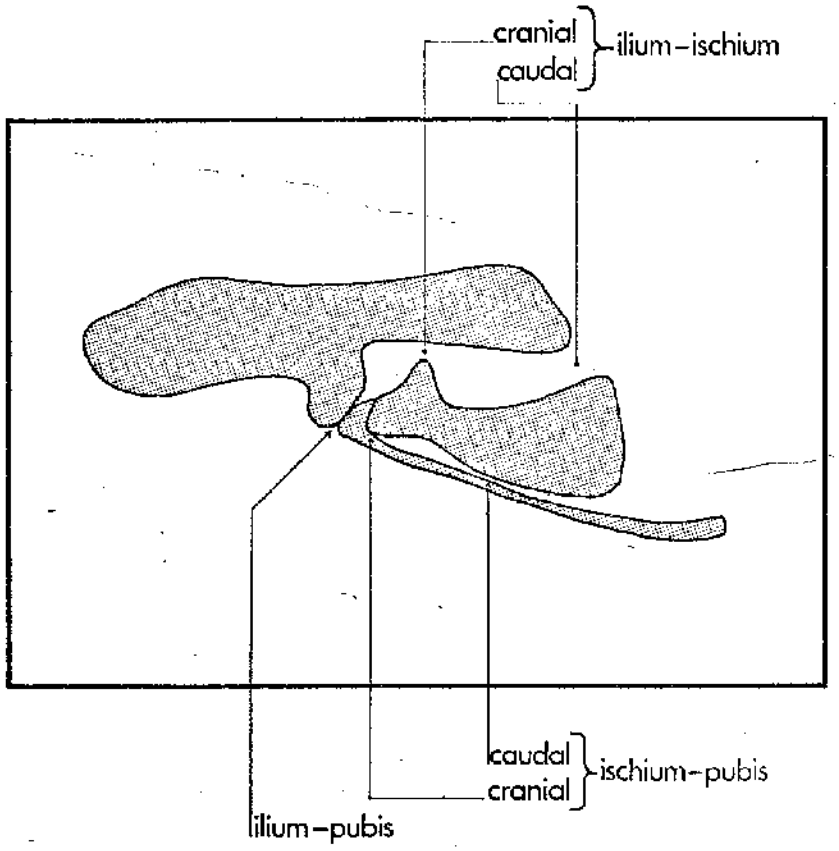


Fig. 84 Tracing of radiograph of os coxae of 70day old bird indicating future sites of fusion

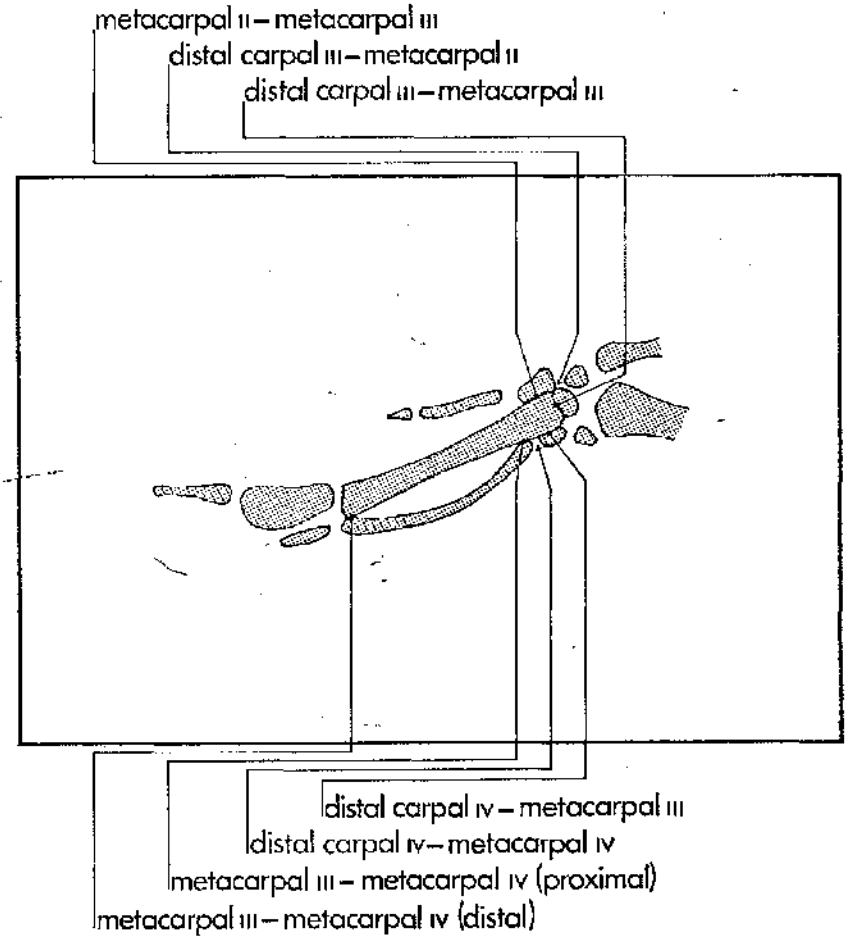


Fig. 85 Tracing of radiograph of wing of 56day old bird indicating future sites of fusion

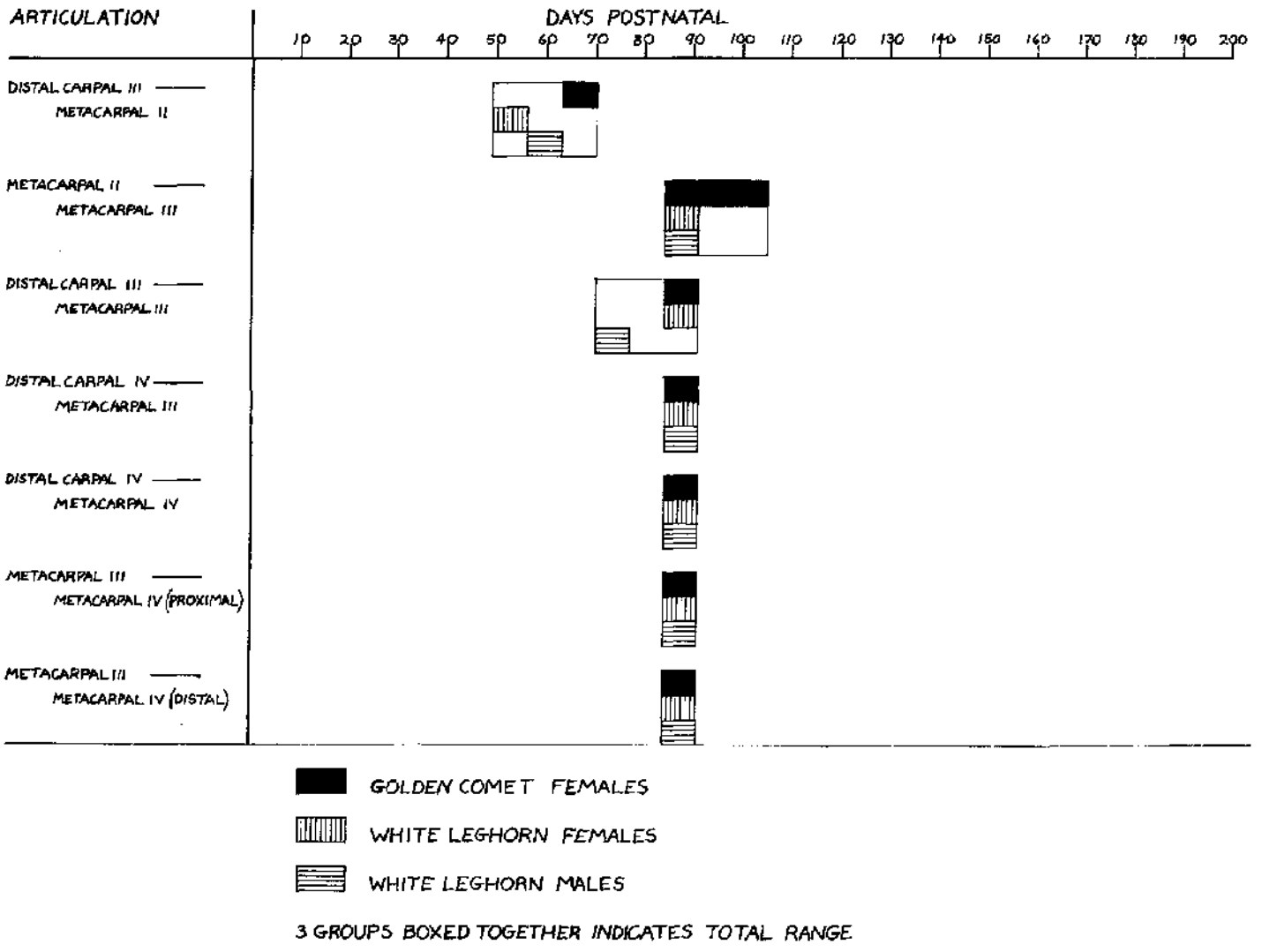


Fig. 86 Range of fusion times of articulations of carpus and metacarpus

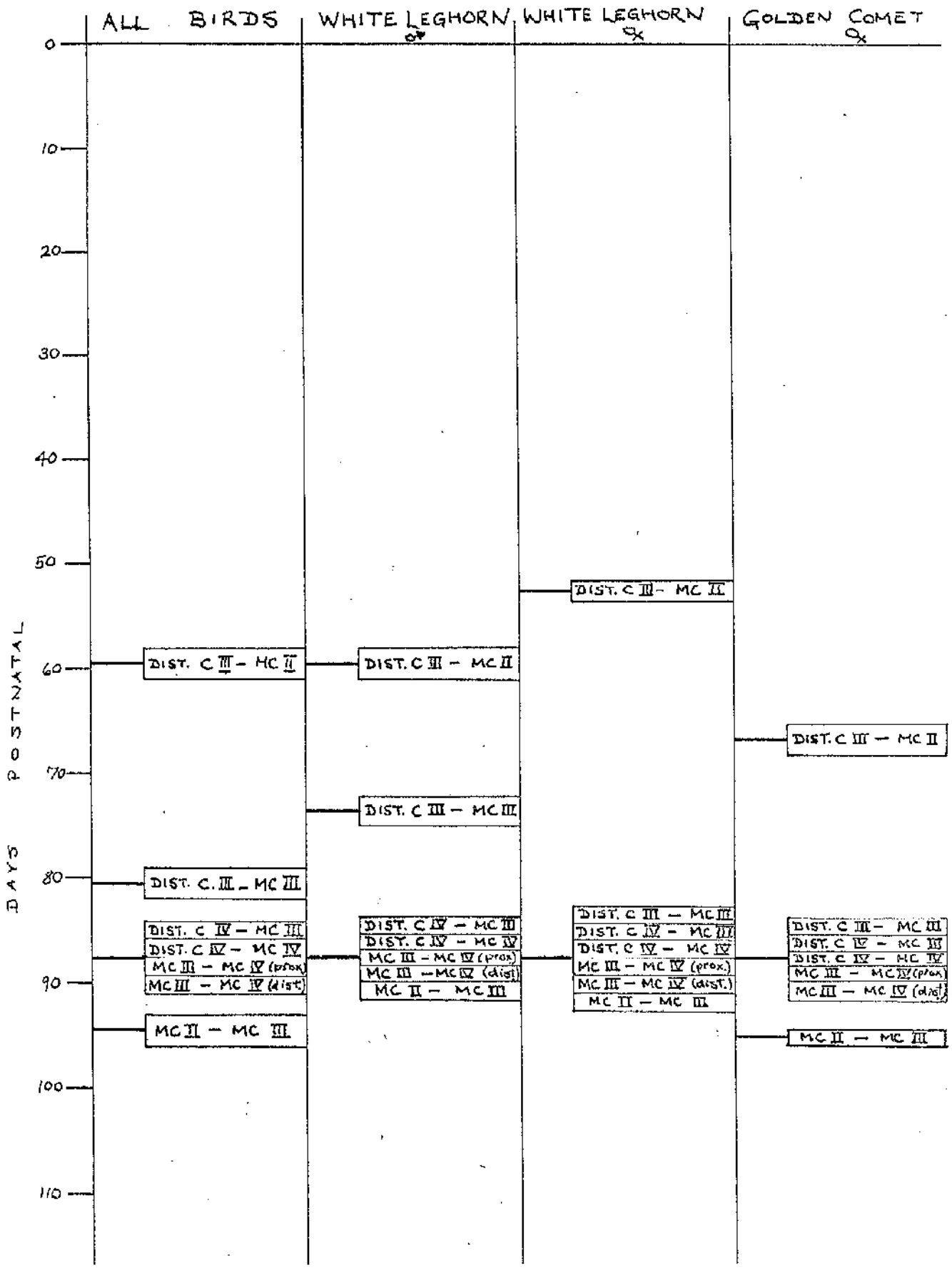


Fig. 87 Sequence of mean fusion times of articulations of carpus and metacarpus.

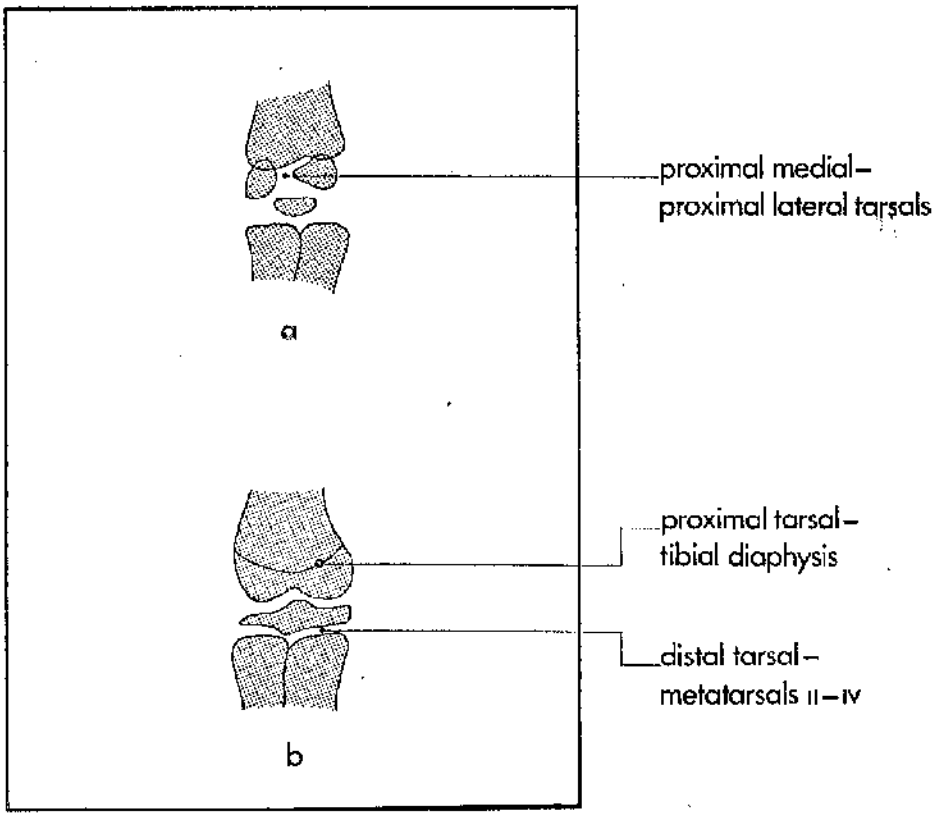


Fig. 88 Tracings of radiographs of tarsus of birds aged a. 42 days and b. 77 days showing future sites of fusion

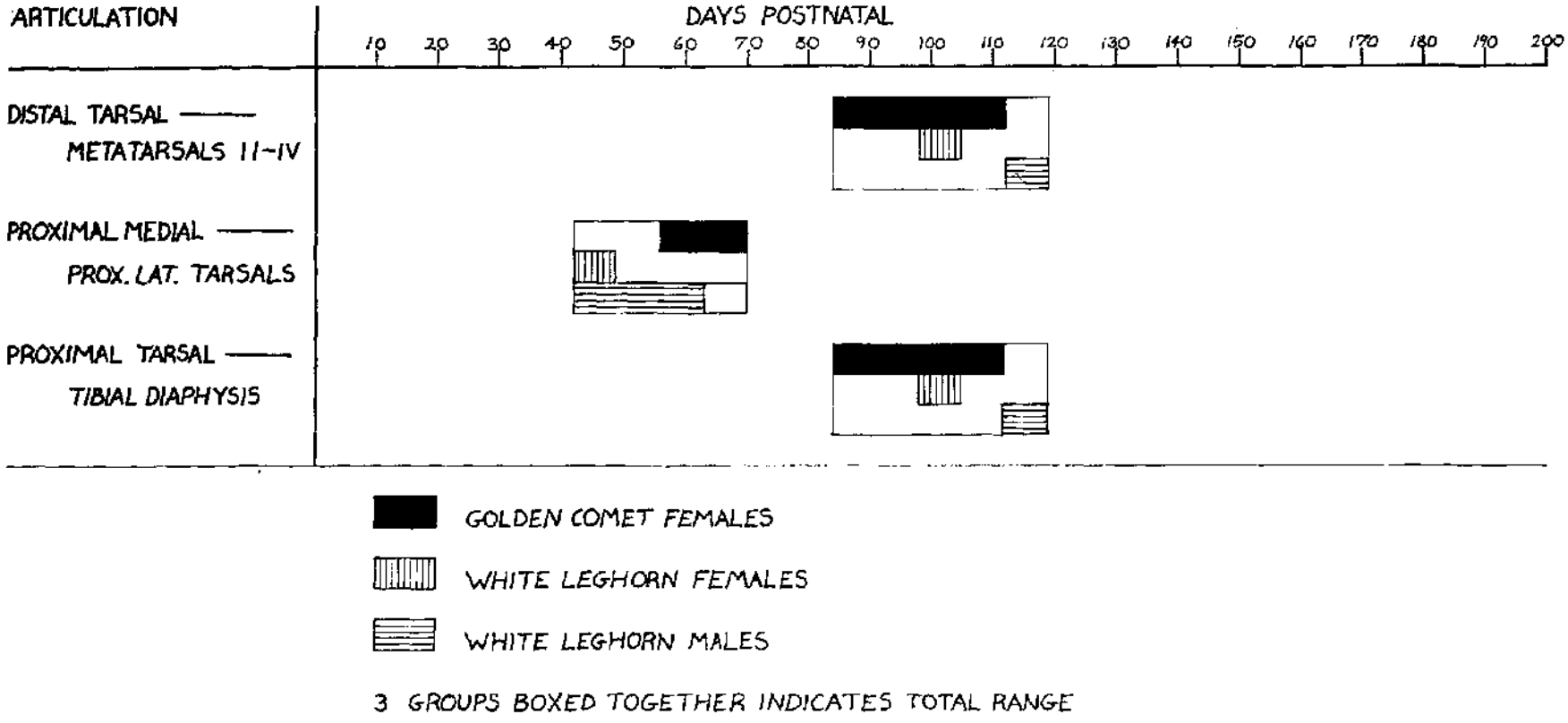


Fig. 89 Range of fusion times of articulations of tarsus

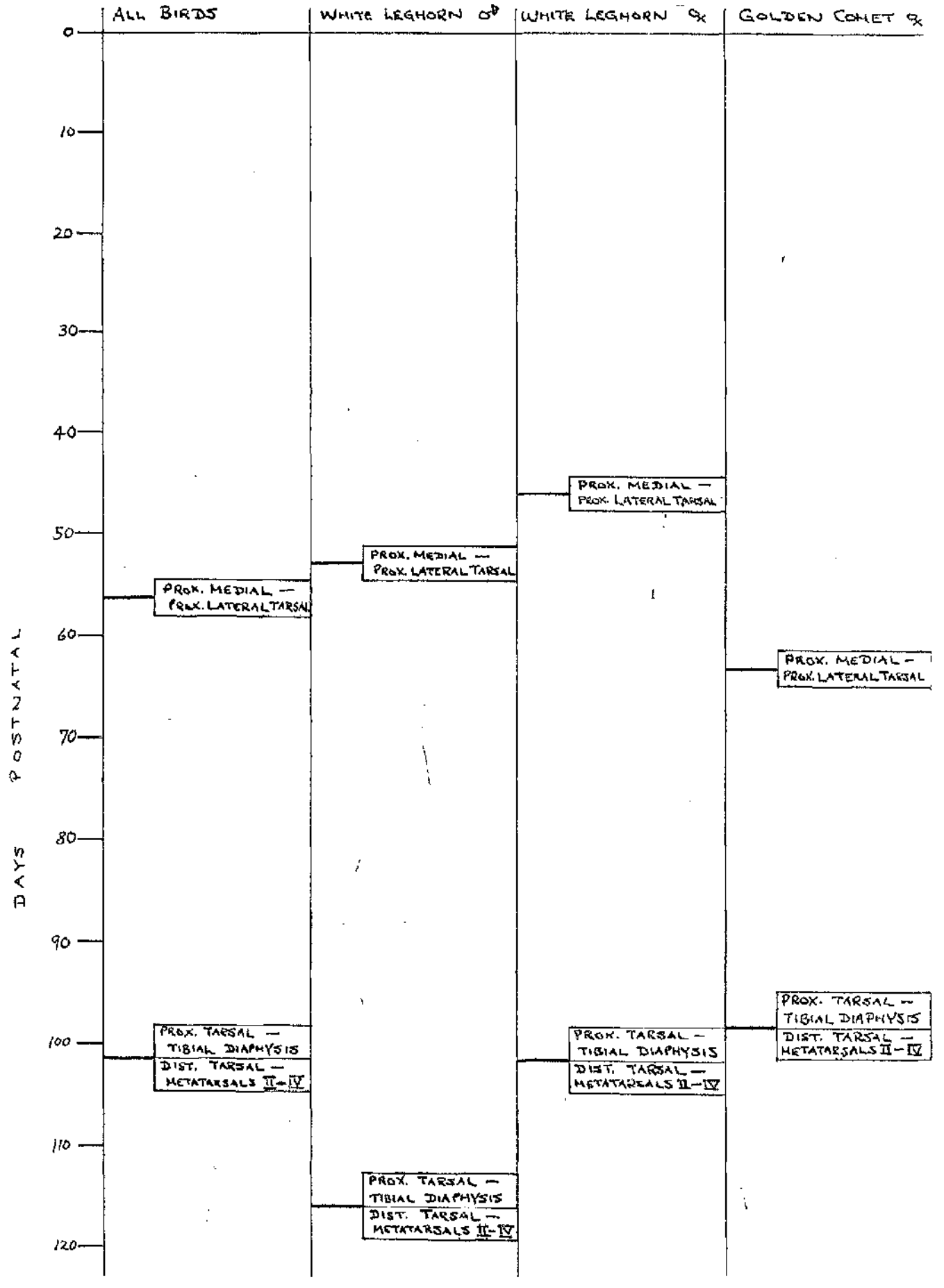


Fig. 90 Sequence of mean fusion times of articulations of tarsus

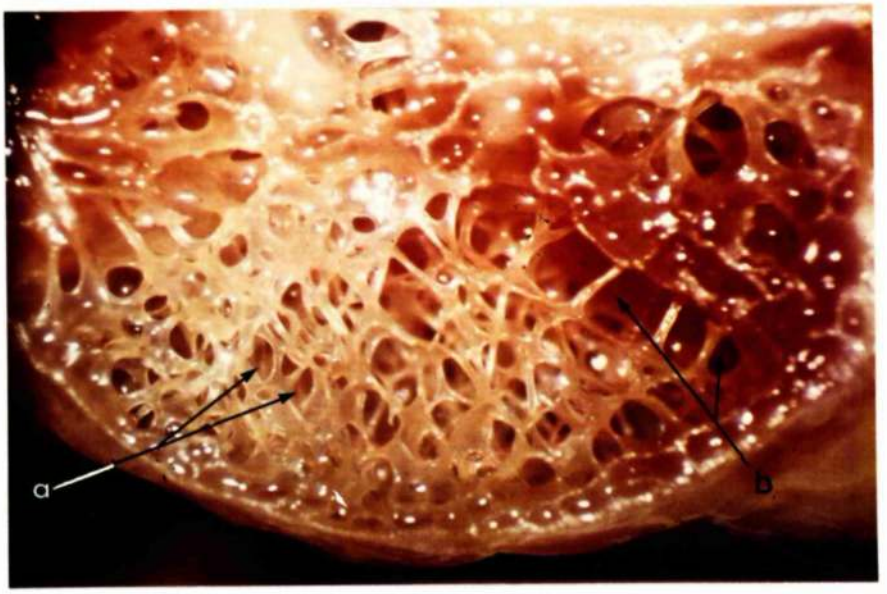


Fig. 91 Parasphenoid/basisphenoid Adult

a. pneumatised spaces

b. spaces filled partially or completely with red marrow

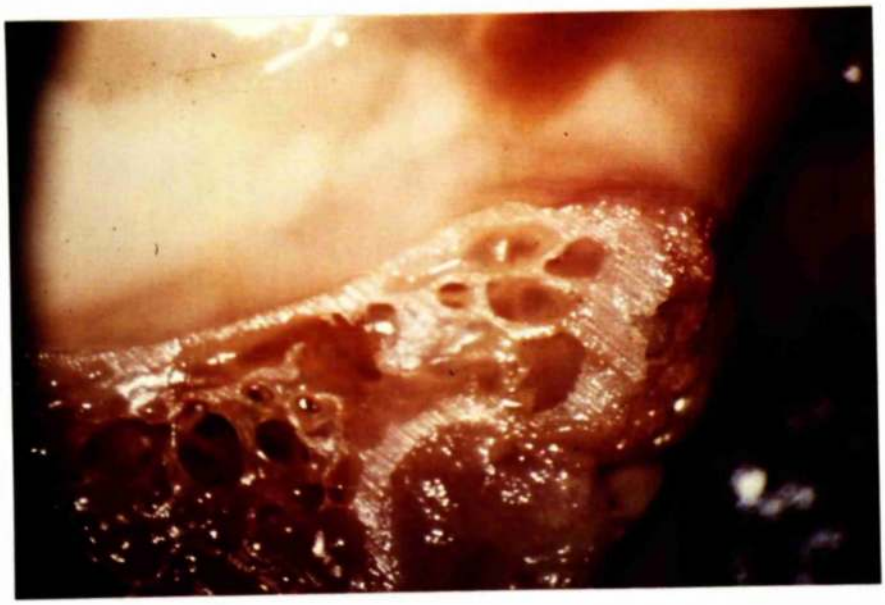


Fig. 92 Basioccipital Adult cockerel
Pneumatisation extends to occipital condyle on right. Marrow persists rostrally.

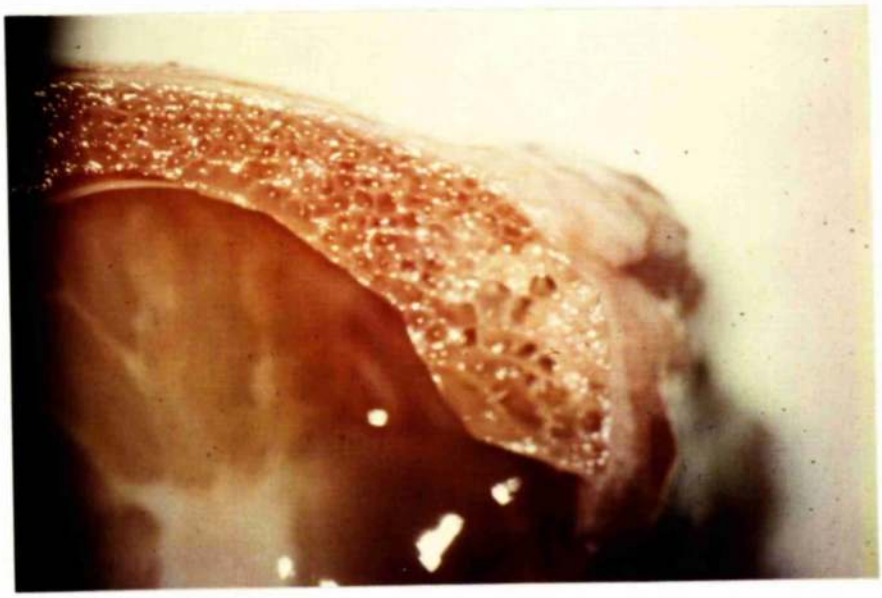


Fig. 93 Supraoccipital and parietal Adult
Pneumatisation is extensive though traces of red marrow are numerous especially in the

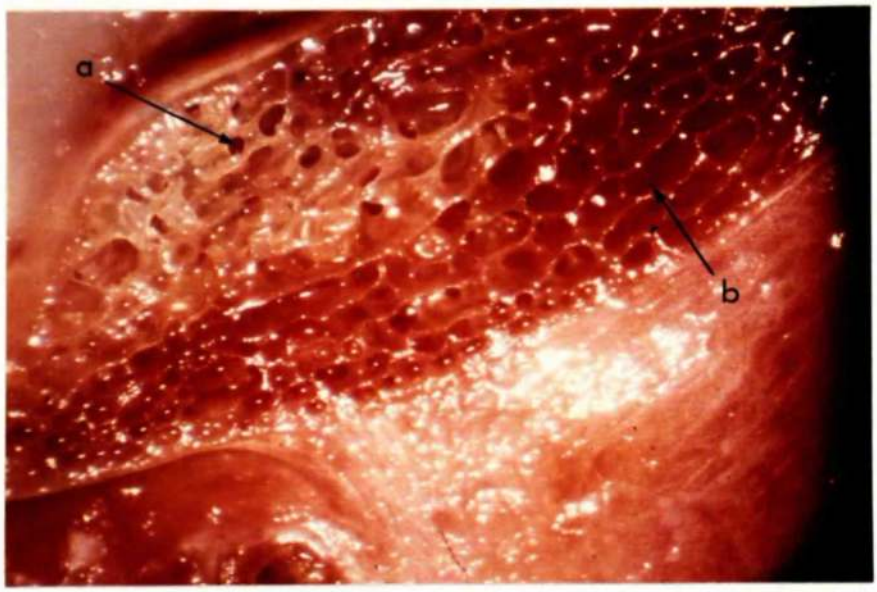


Fig. 94 Frontal Adult

a. pneumatised spaces

b. marrow-filled spaces

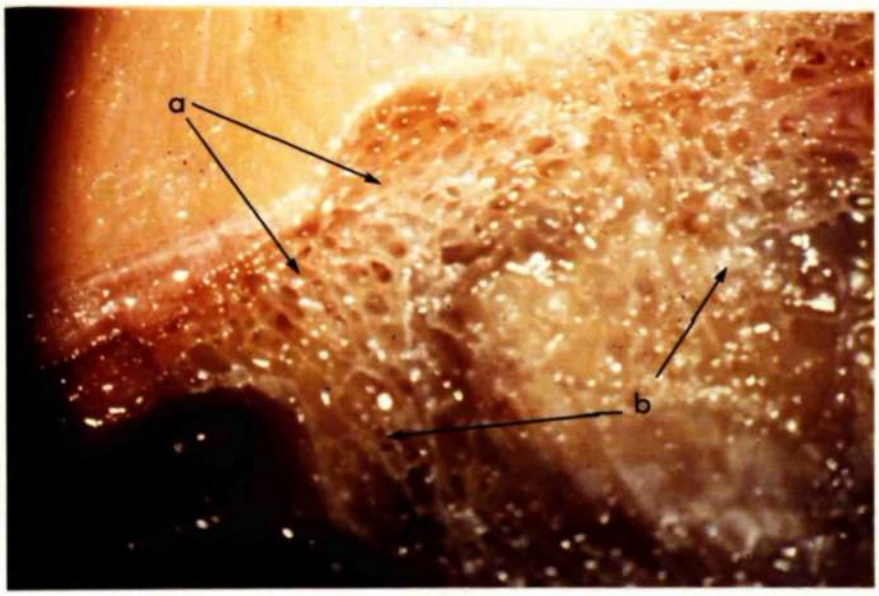


Fig. 95 Frontal and mesethmoid Adult

Midsagittal section showing extensive pneumatisation in a. frontal and b. mesethmoid where it forms part of interorbital septum

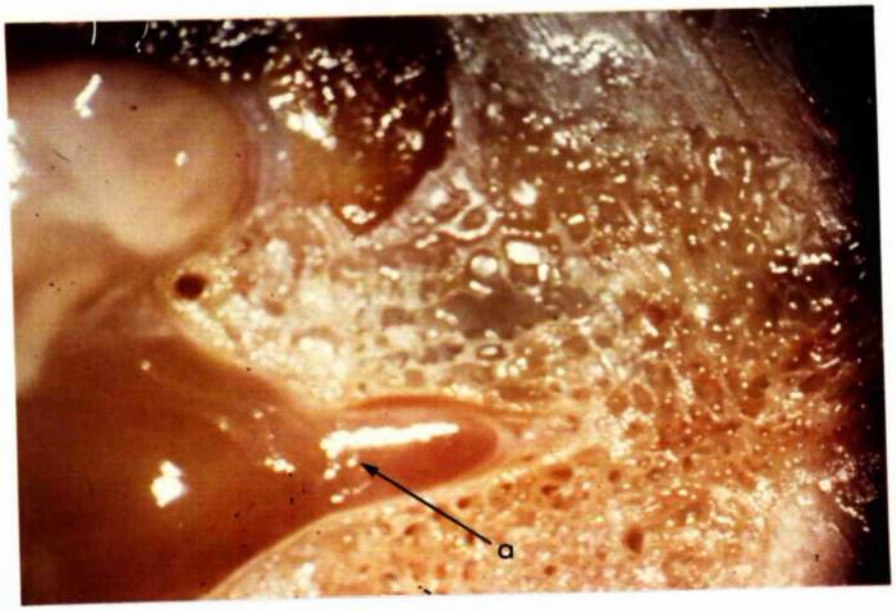


Fig. 96 Mesethmoid, orbitosphenoid and parasphenoid/basisphenoid Adult

Midsagittal section showing a. optic chiasma. Dorsally is orbitosphenoid fused rostrally (to the right) with the mesethmoid forming caudal part of interorbital septum. Ventrally is rostrum of parasphenoid. All show extensive pneumatisation.

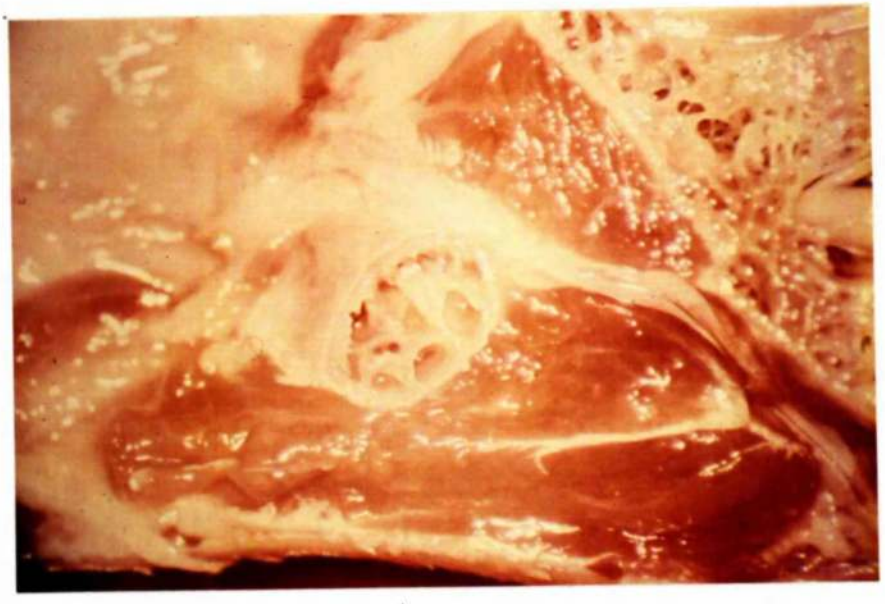


Fig. 97 Pterygoid Adult
Transverse section (in centre of field)
with full pneumatisation

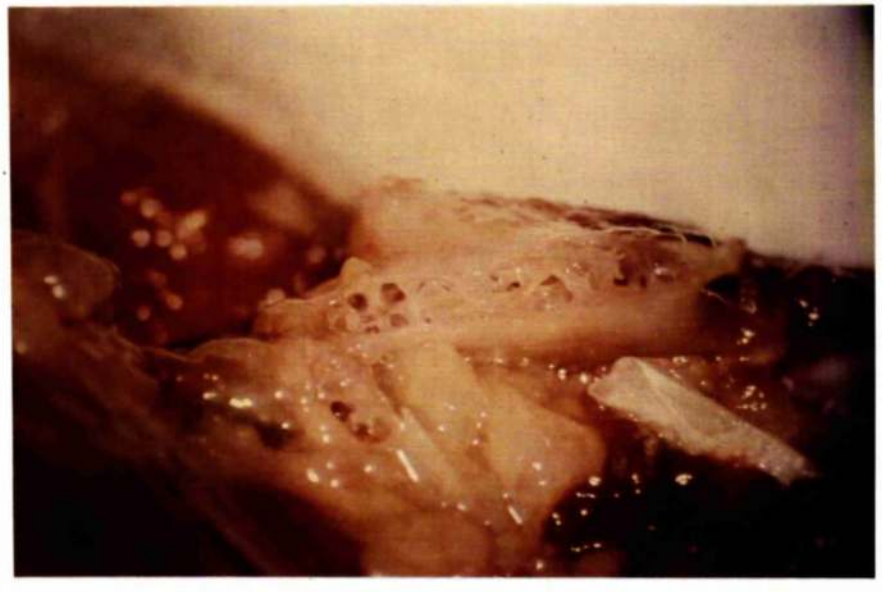


Fig. 98 Quadrate Adult
Longitudinal section showing complete
pneumatisation

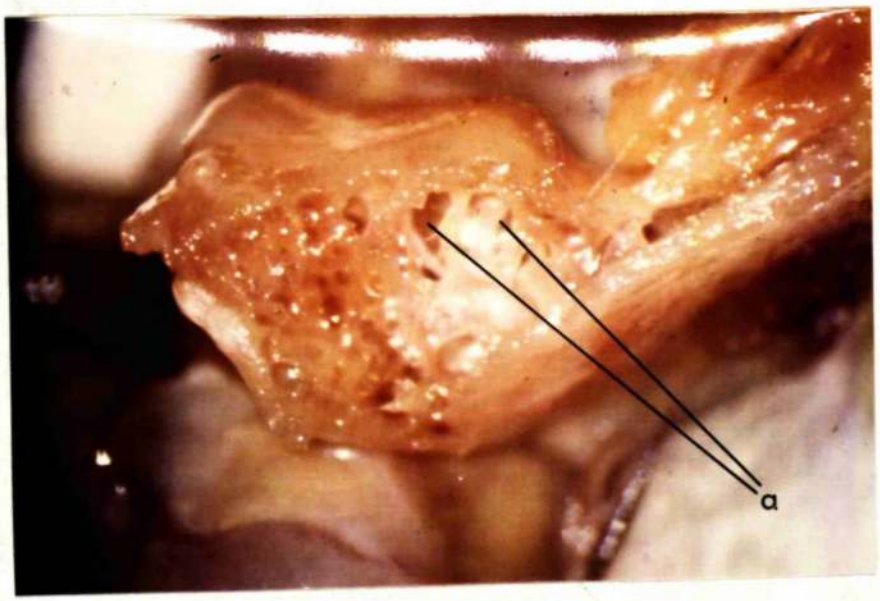


Fig. 99 Mandible Adult

Transverse section through proximal part showing pneumatised spaces marked a.

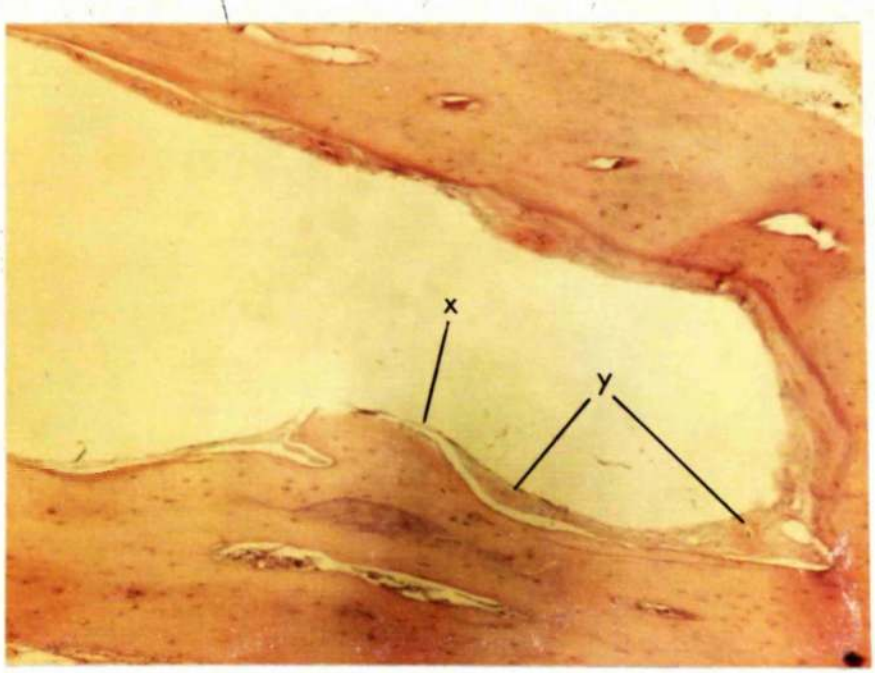


Fig. 100 Pterygoid Adult

H&E X40

Single air-filled cavity in centre surrounded by squamous epithelium (x). Lamina propria thickened around numerous blood vessels (y).

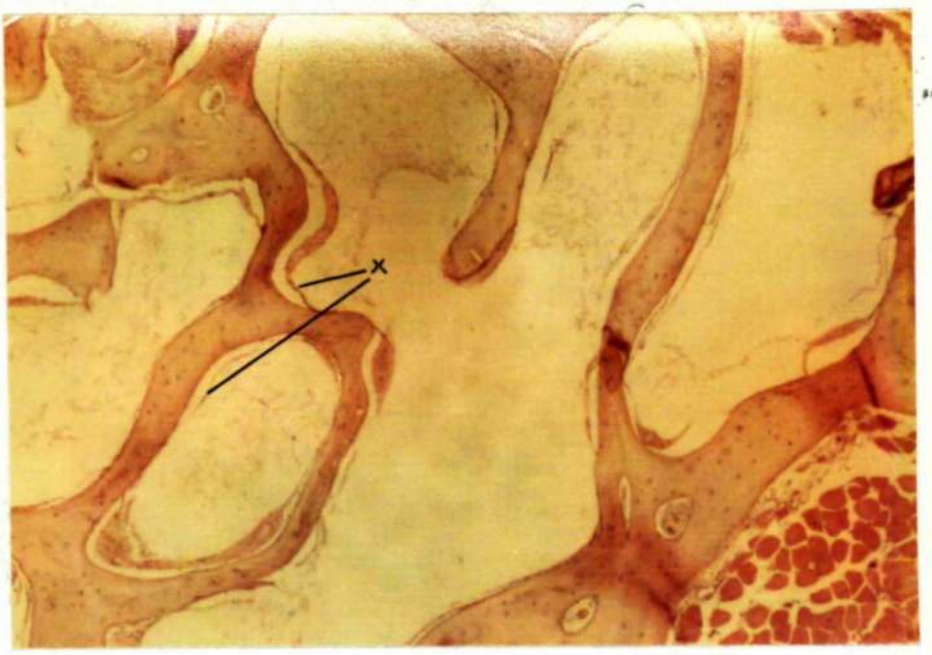


Fig. 101 Prefrontal Adult
H&E X40

Pneumatisation widespread in numerous small inter-communicating spaces enclosed by spicules of cancellous bone and lined by squamous epithelium (x).

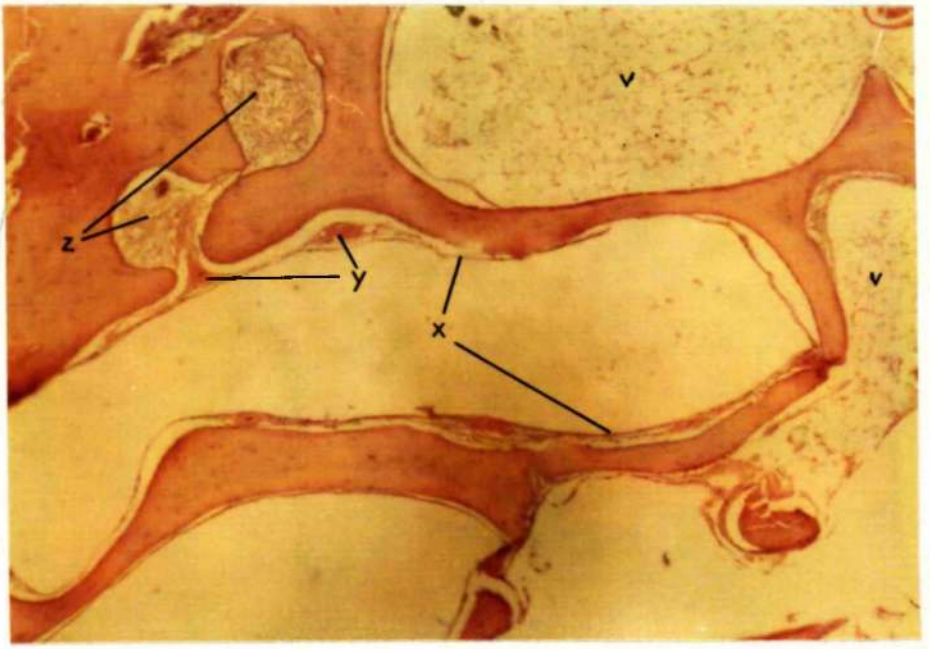


Fig. 102 Mandible Adult
H&E X40

Squamous epithelium (x) underlain by lamina propria thickened around blood vessels (y) and continuous with fibrous tissue filling spaces (z). Mucus material seen in some pneumatised spaces (v).

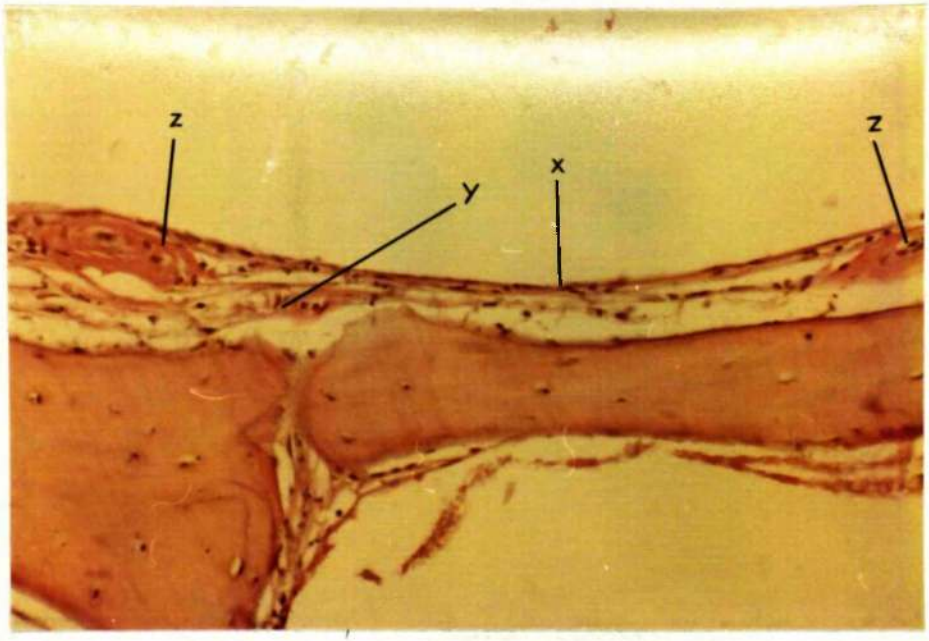


Fig. 103 Mandible Adult
H&E X100
Squamous epithelium (x), lamina propria (y) and numerous blood vessels (z).

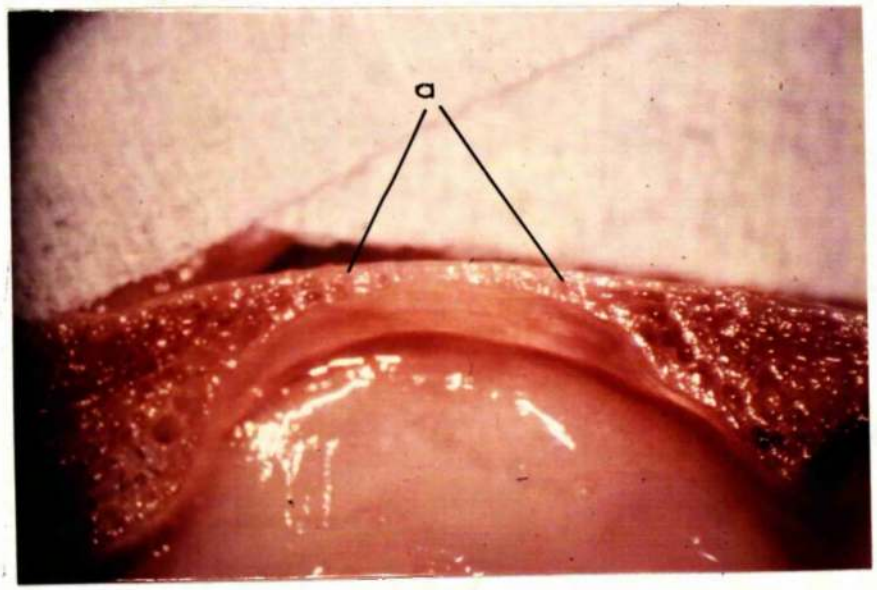


Fig. 104 Frontal and parietal Adult
Sagittal section showing frontal on left and parietal on right. a indicates limits of region of frontal lacking diploe and pneumatisatio

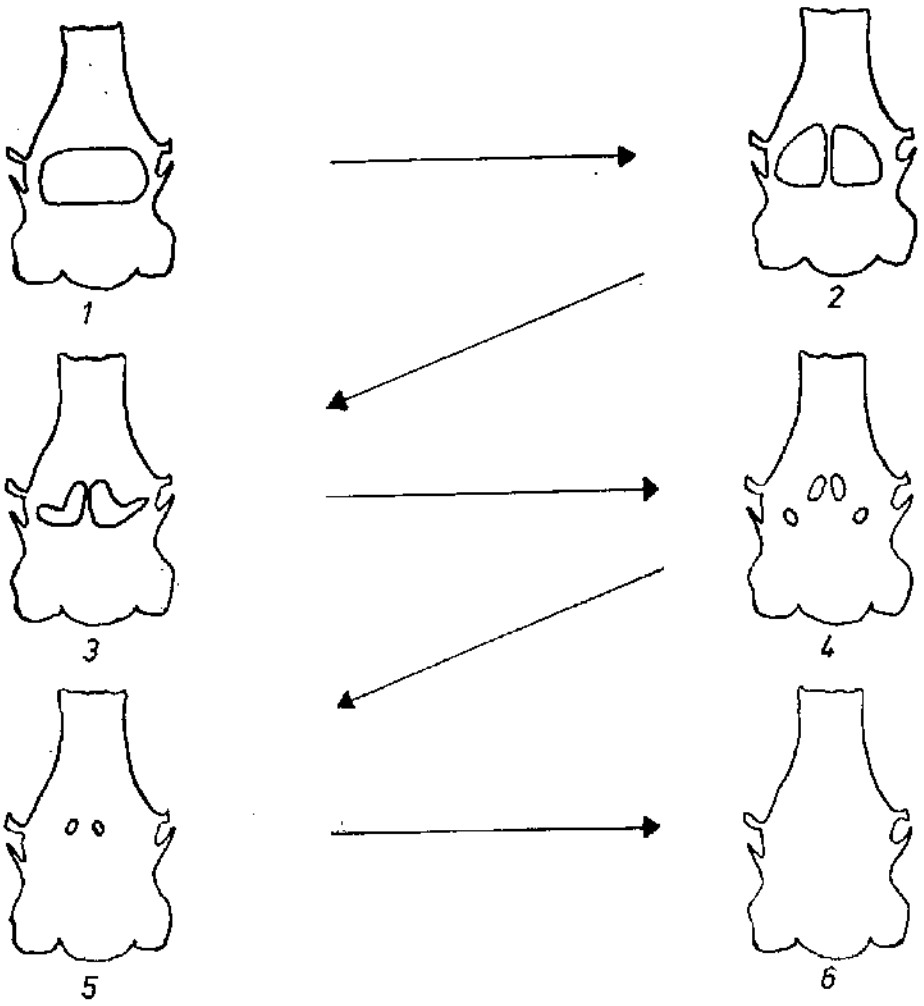


Fig. 105 Stages of cranial pneumatisation in adult determined by transillumination

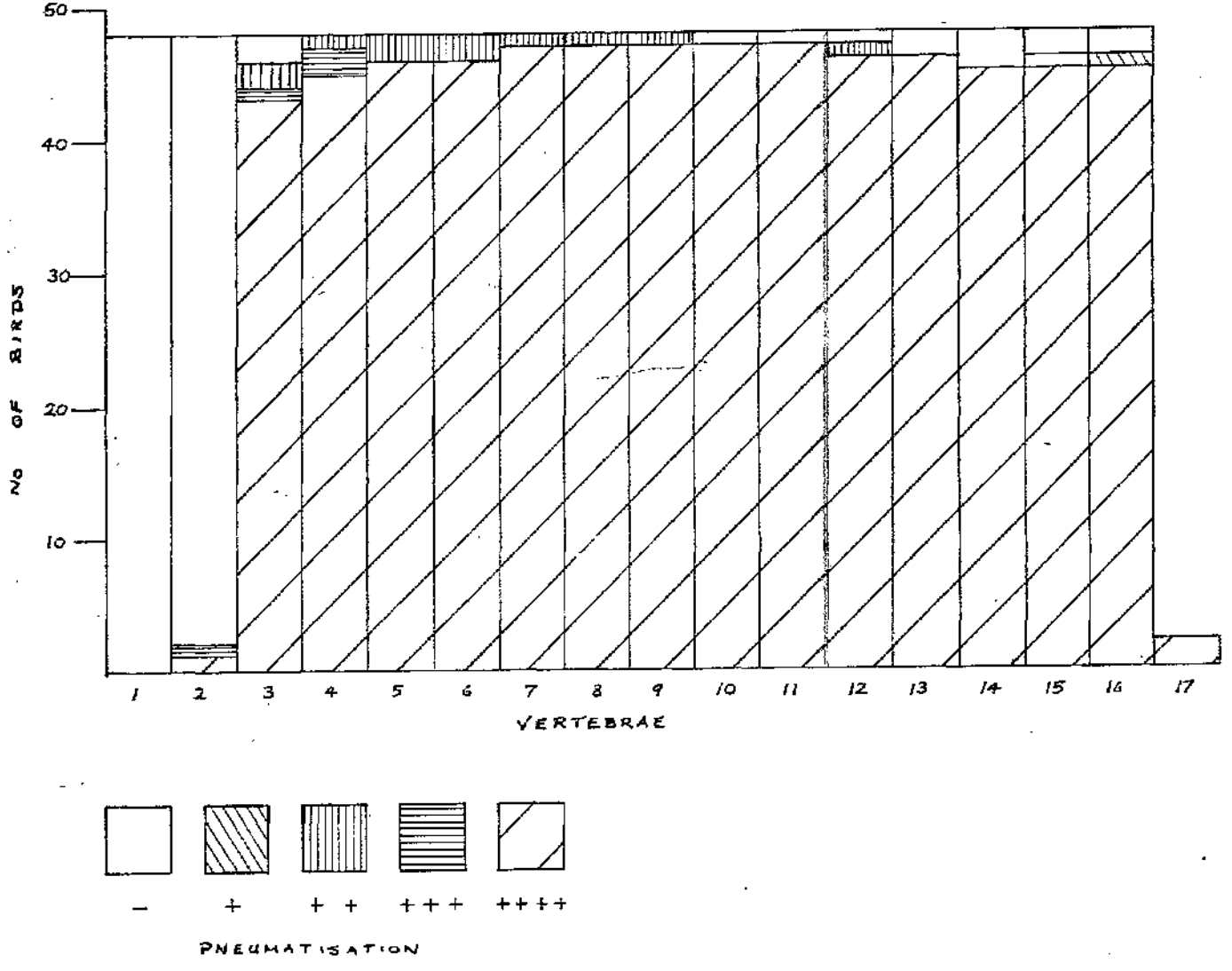


Fig. 106 Degree of pneumatisation in cervical vertebrae - hens

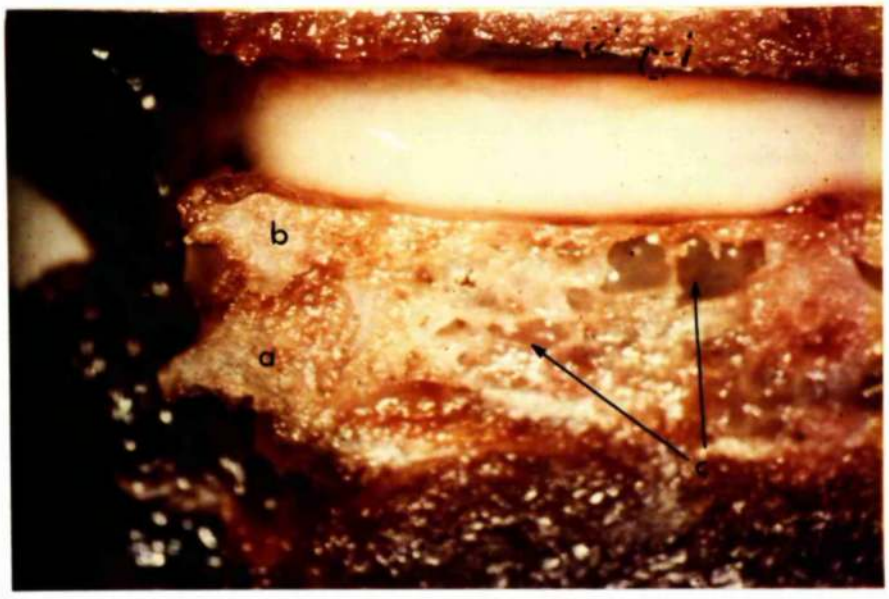


Fig. 107 Atlas and axis Adult

Midsagittal section showing

a. atlas

b. odontoid process of axis

c. pneumatised spaces in body of axis

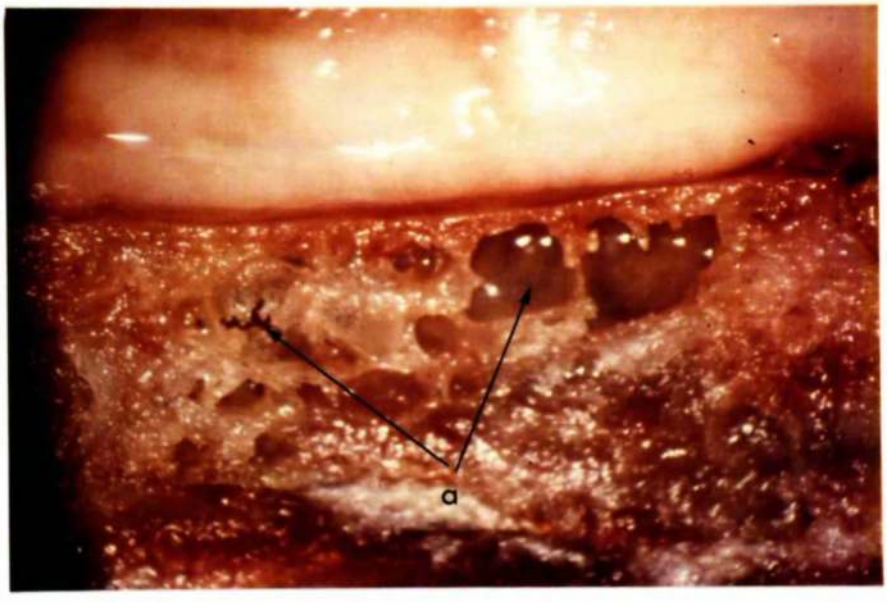


Fig. 108 Axis Adult

Midsagittal section showing pneumatised spaces marked a

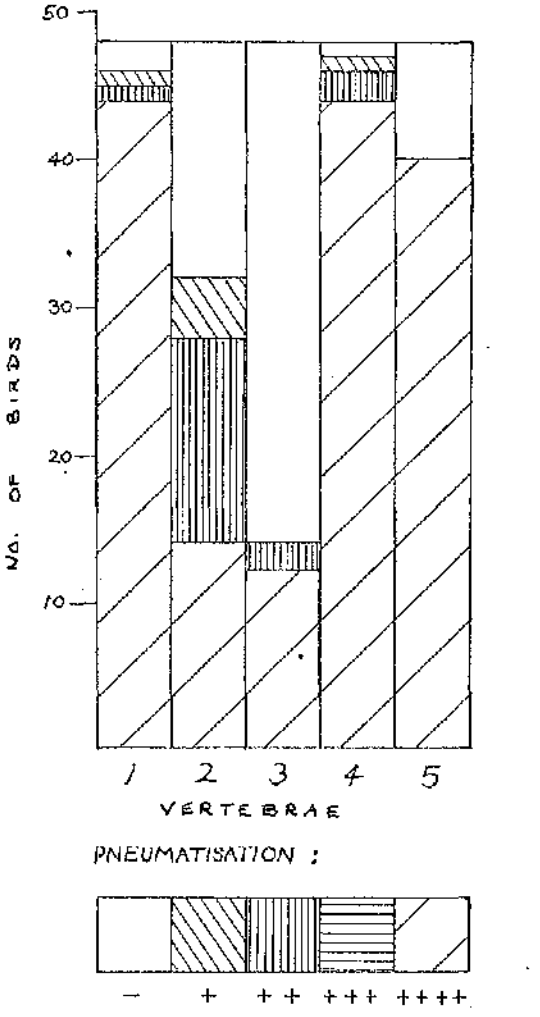


Fig. 109 Degree of pneumatisation in thoracic vertebrae - hens

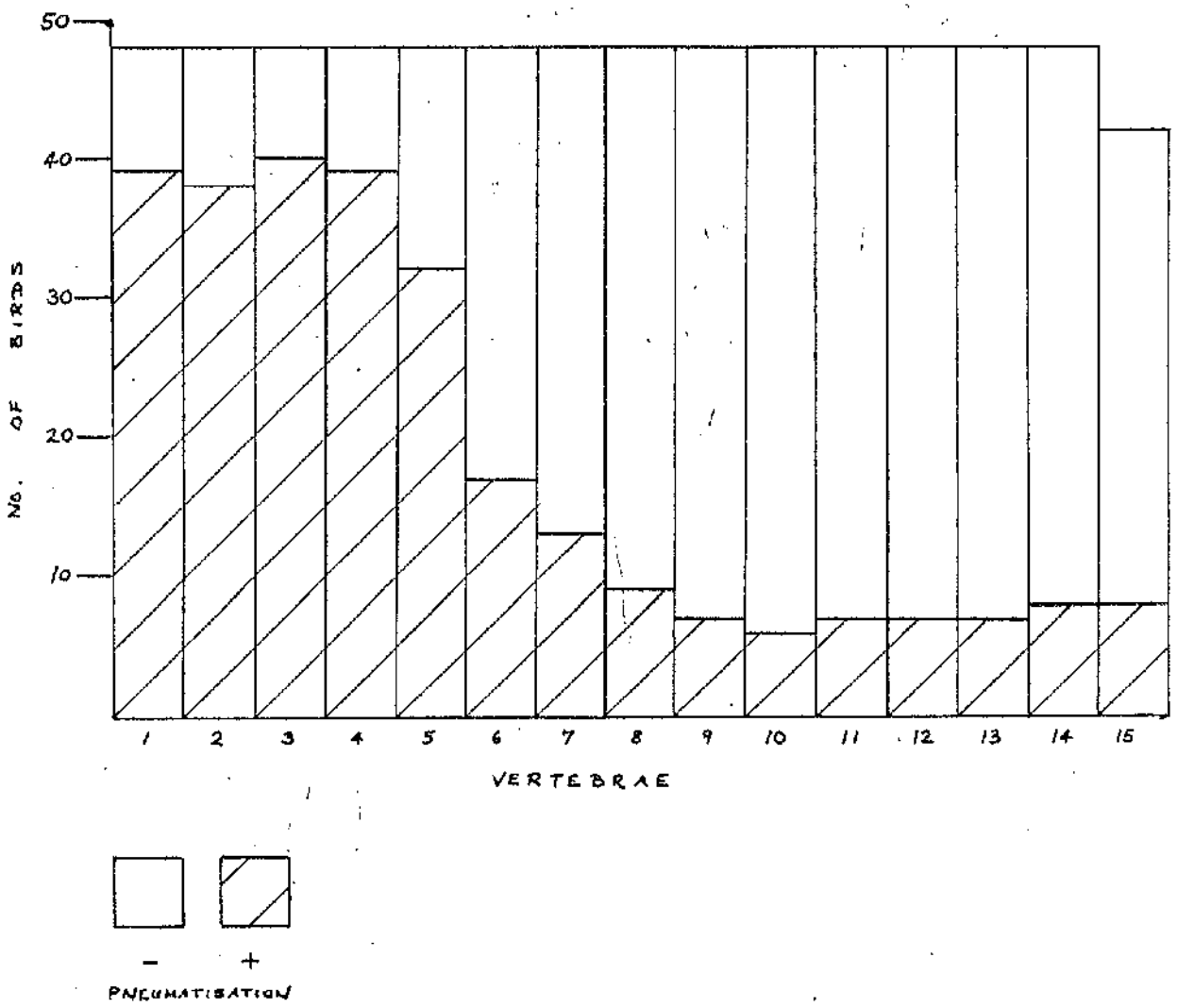


Fig. 110 Occurrence of pneumatisation in lumbosacral vertebrae - hens

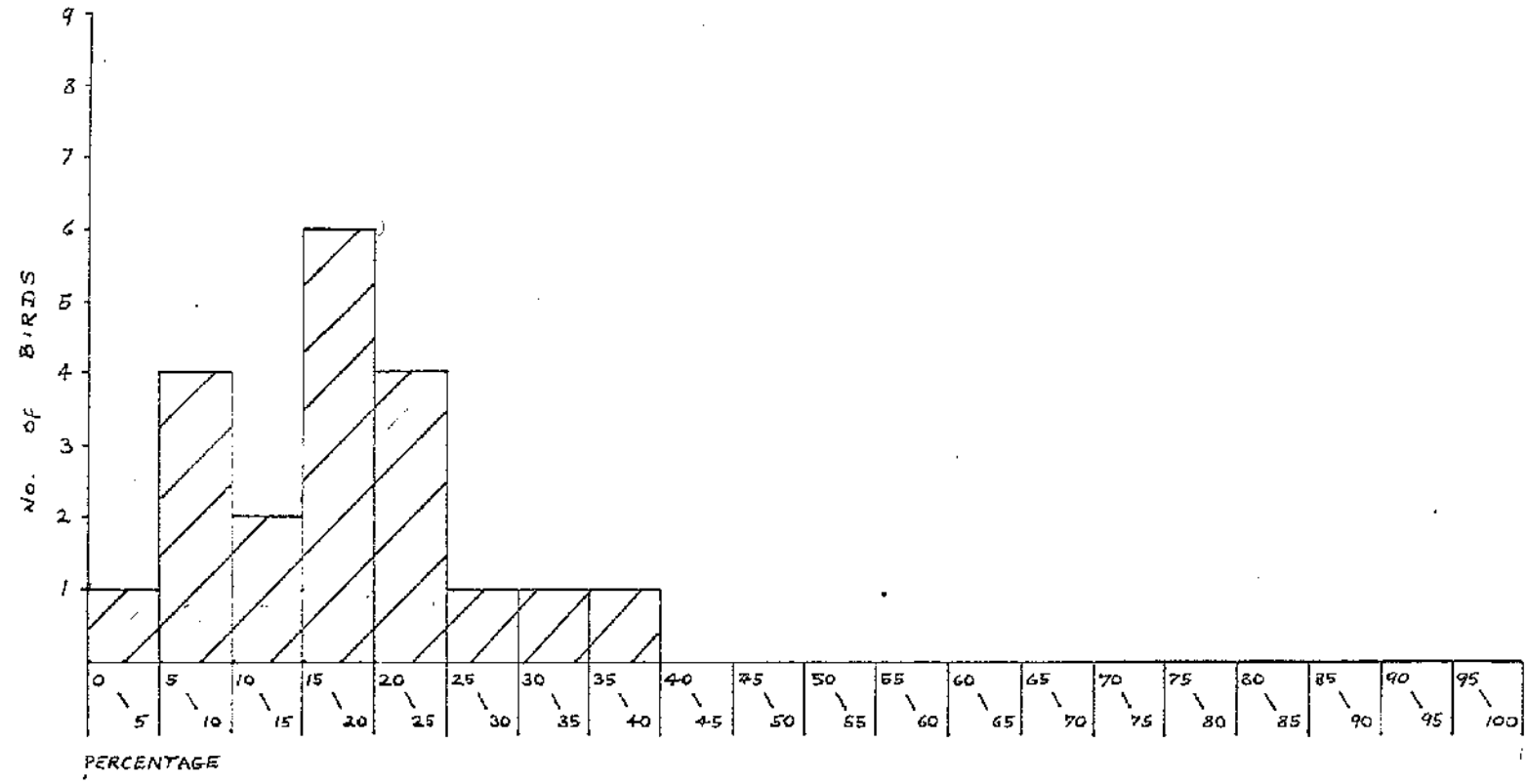


Fig. III Extent of pneumatisation in body and keel of sternum - hens

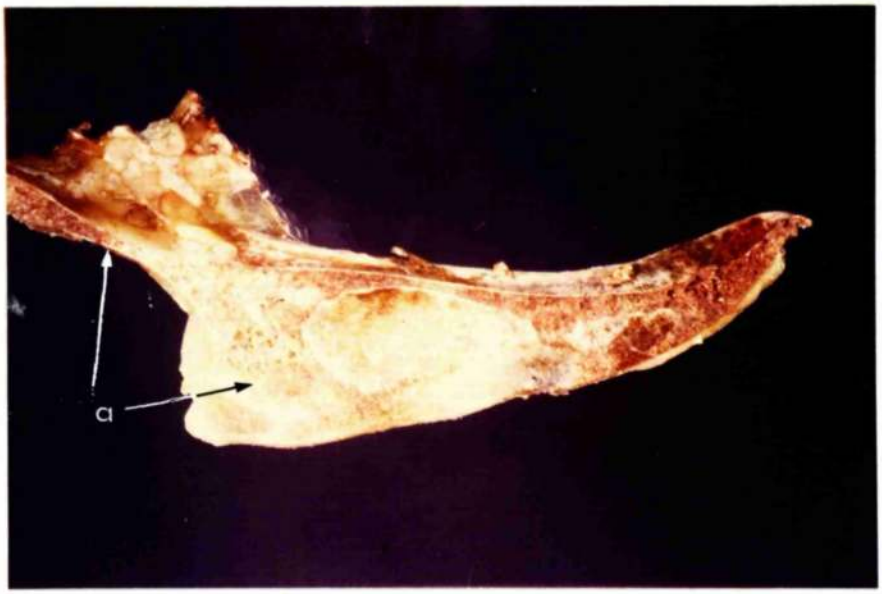


Fig. 112 Sternum Adult

Midsagittal section

a. pneumatisation in body and extending into rostral part of keel.

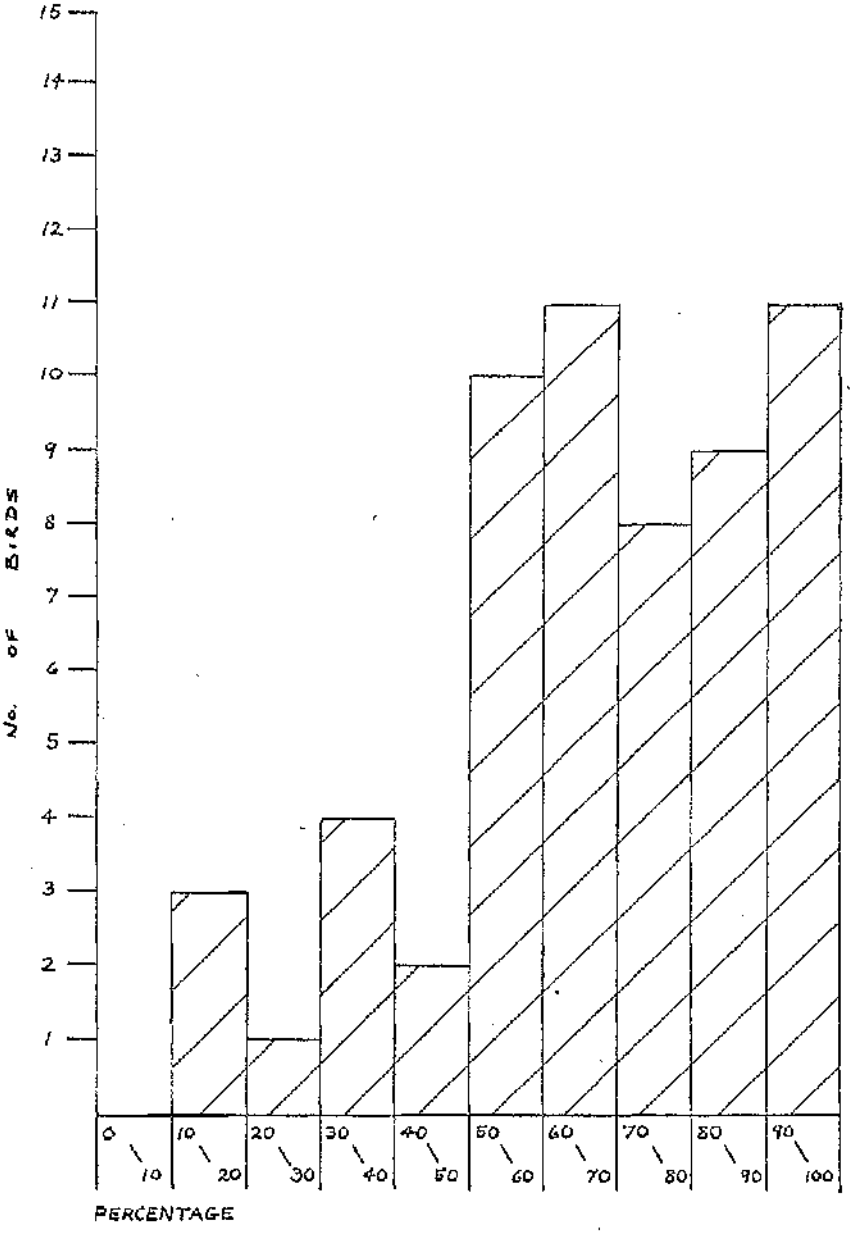


Fig. 113 Extent of pneumatisation in humeri - hens

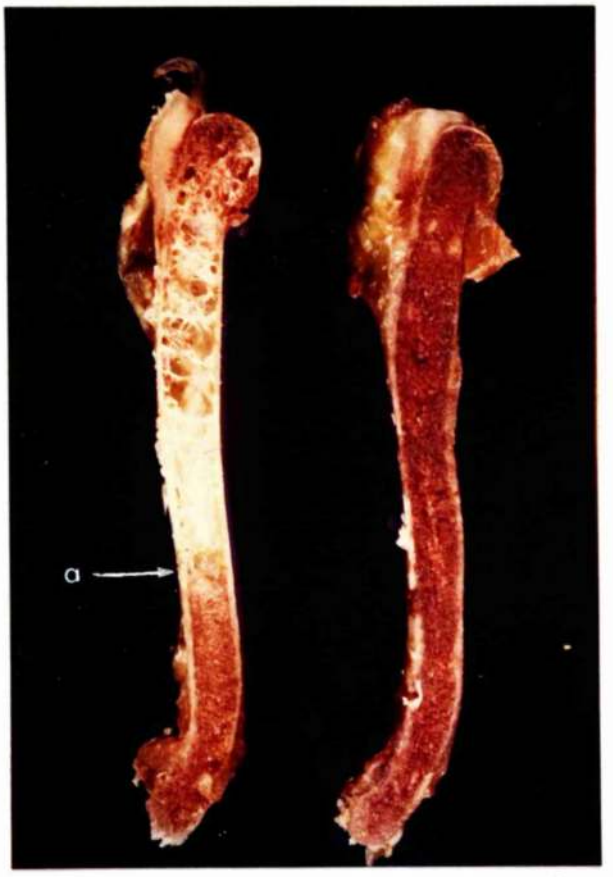


Fig. 114 Left and right humeri Adult
In the left pneumatisation extends to level a, but is completely absent on right.

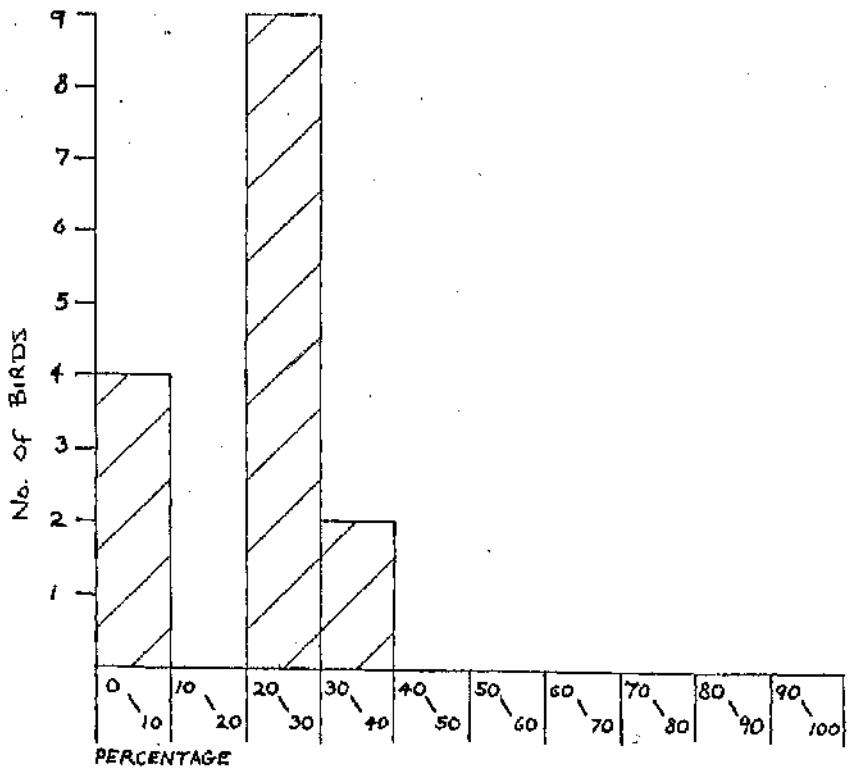


Fig. 115 Extent of pneumatisation in coracoids - hens

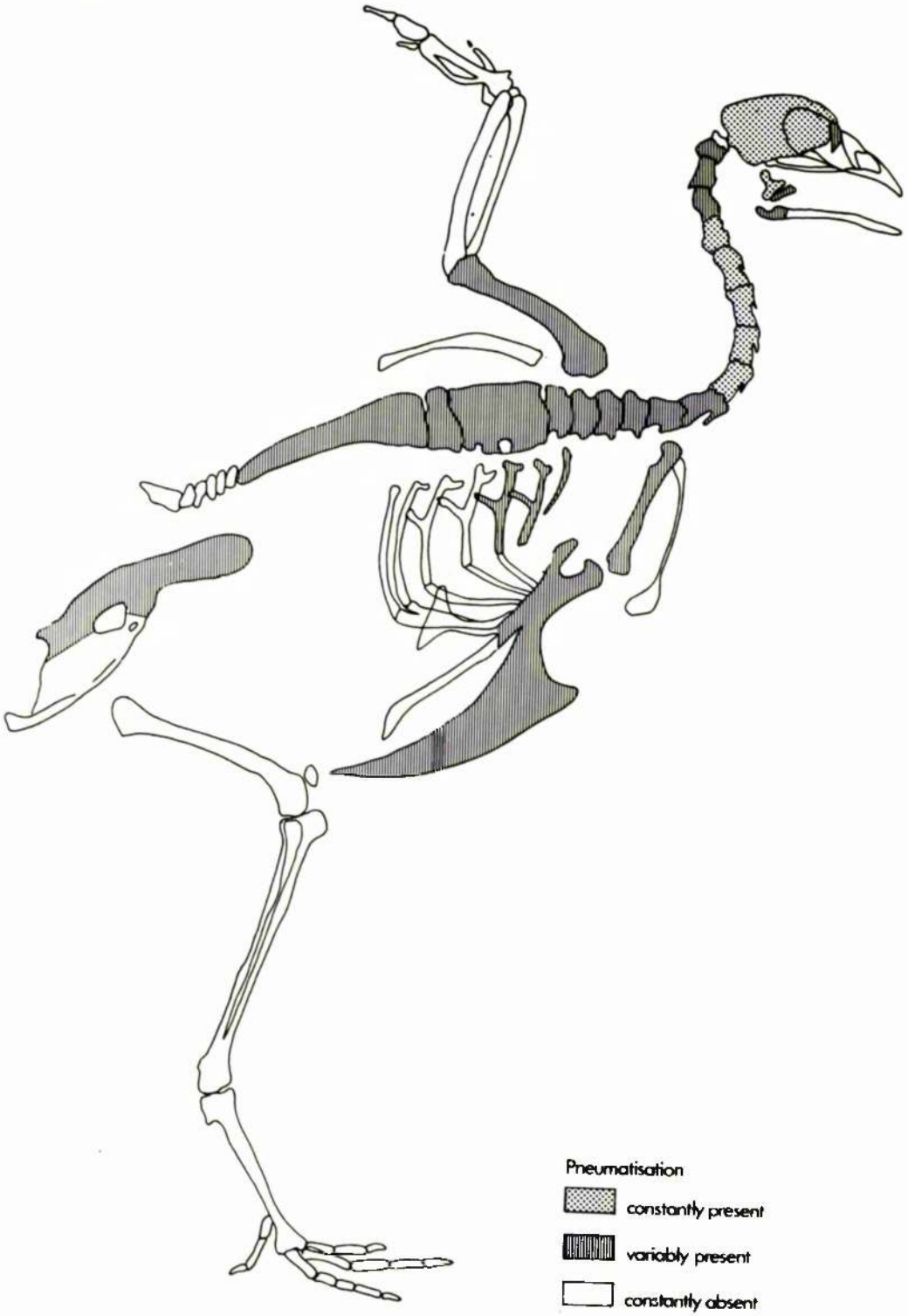


Fig. 116 Occurrence of pneumatisation in whole skeleton

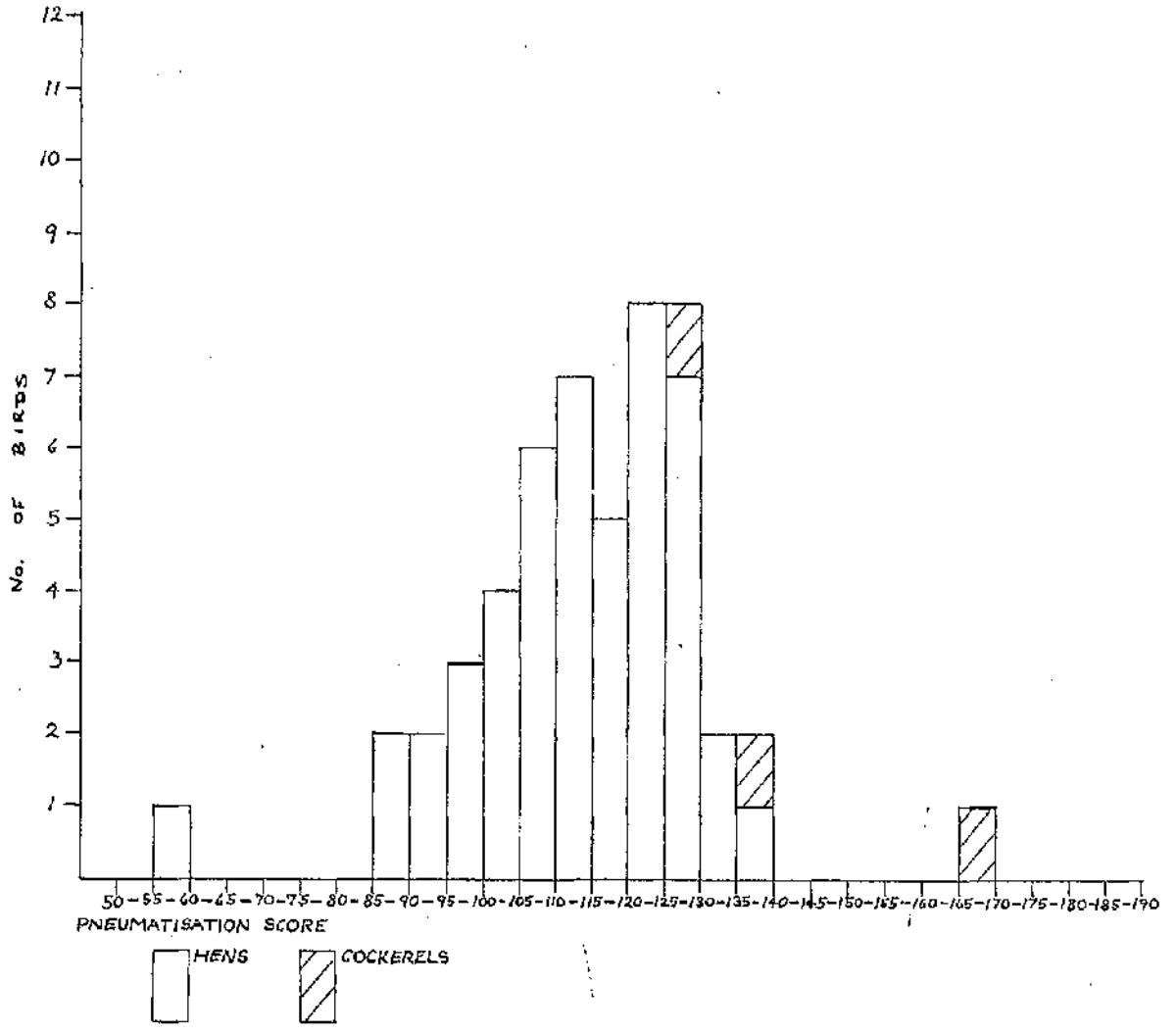


Fig. 117 Histogram of pneumatisation scores of adult Golden Comets.

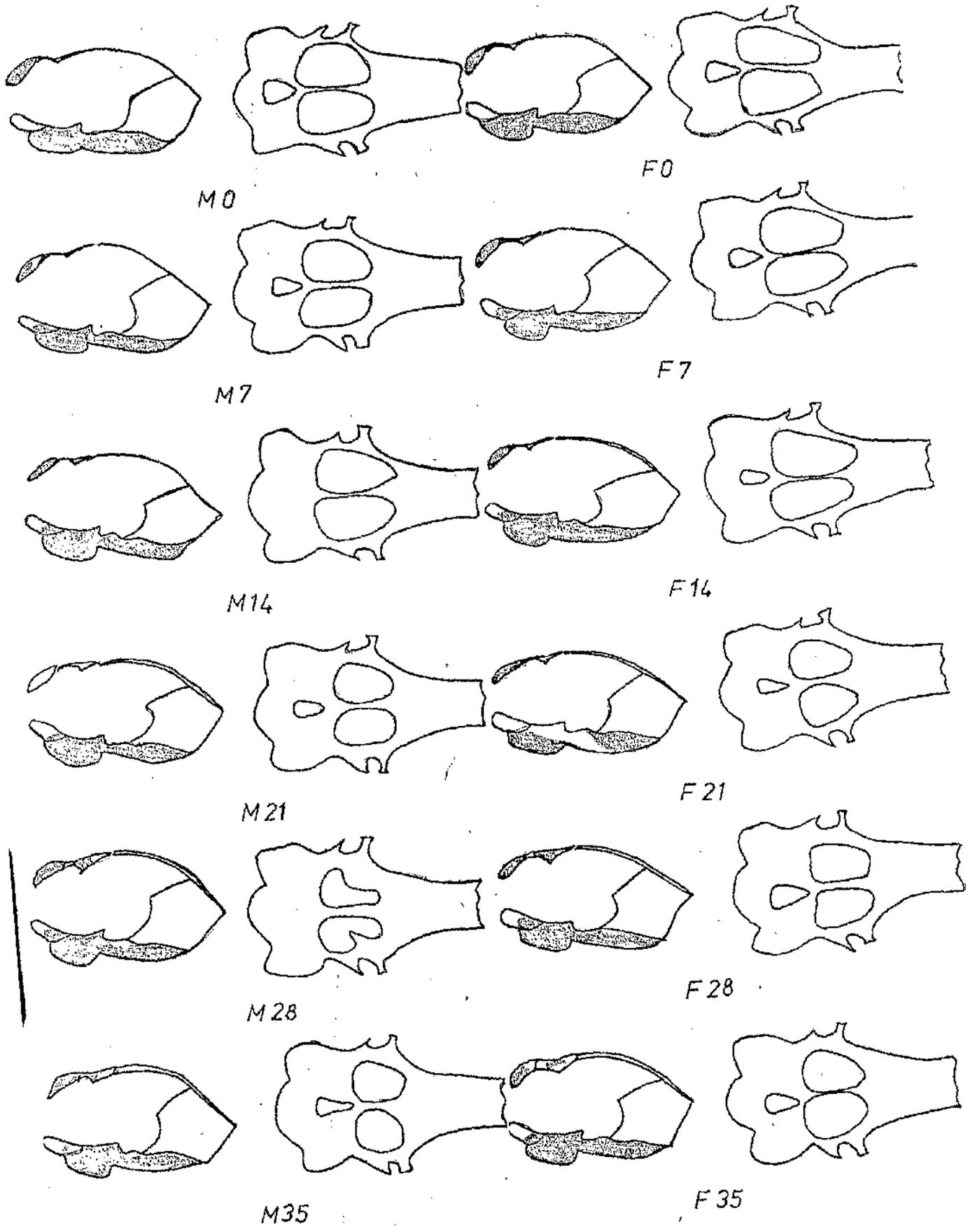


Fig. 118a

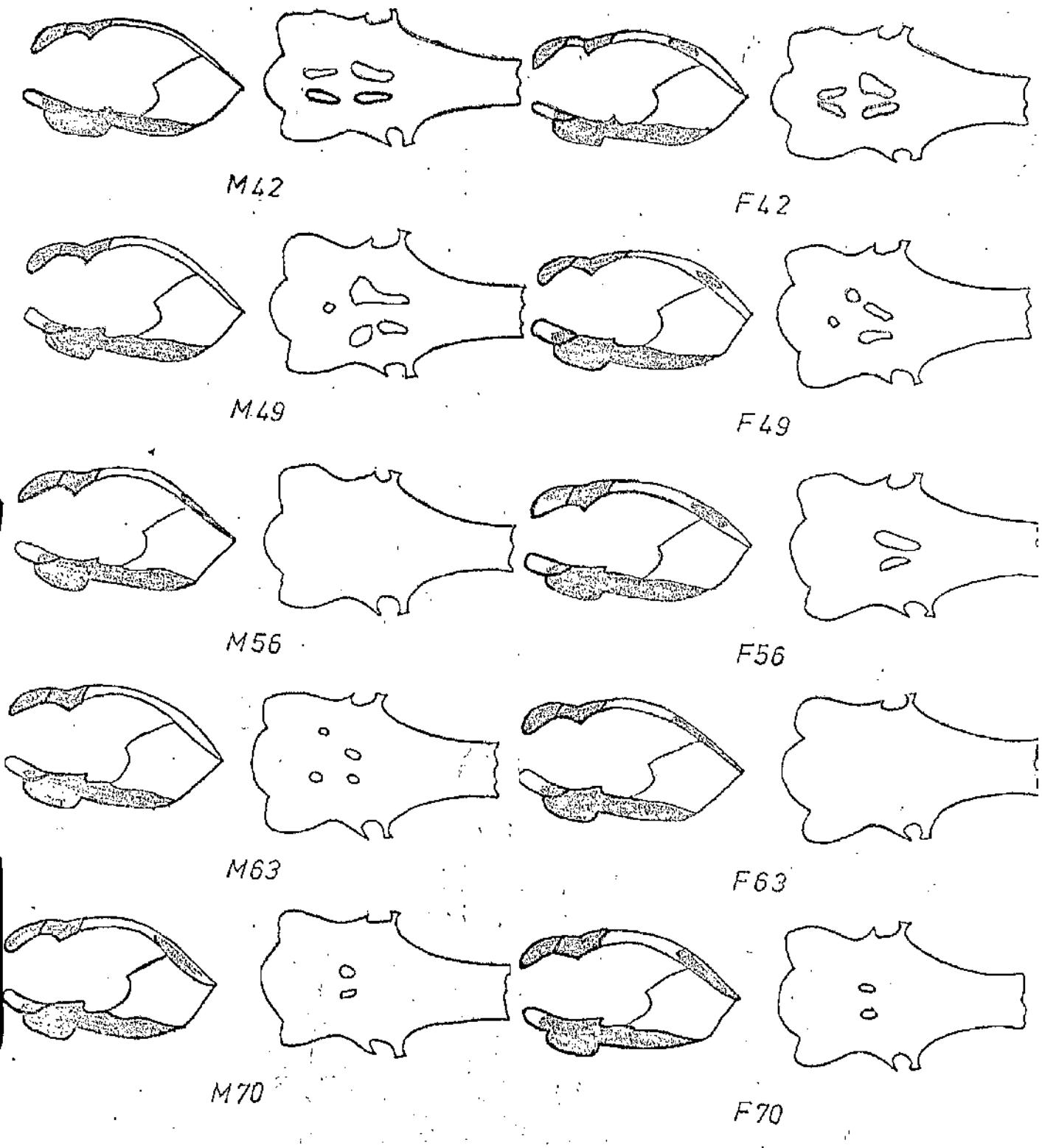


Fig. 118b

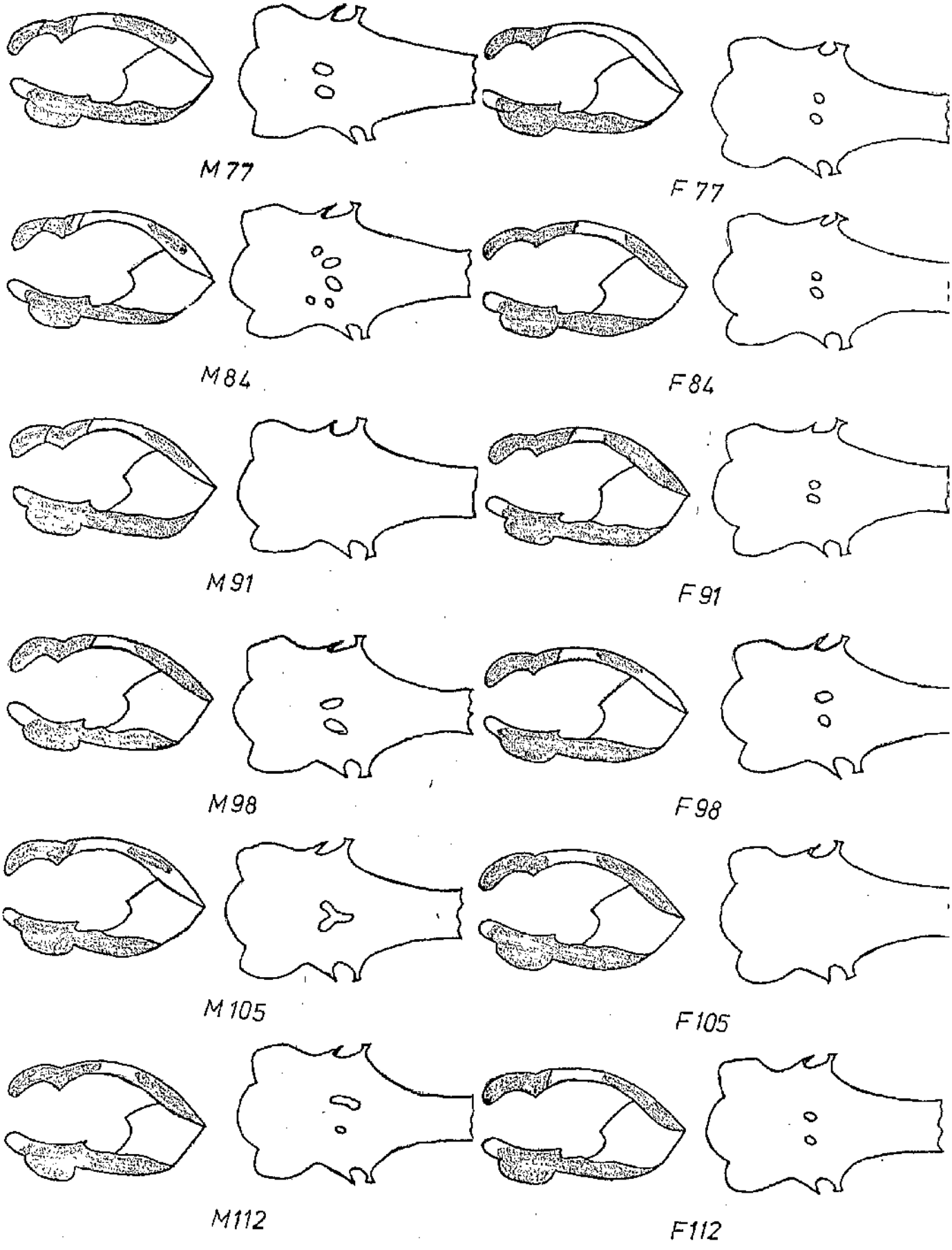


Fig. 118c

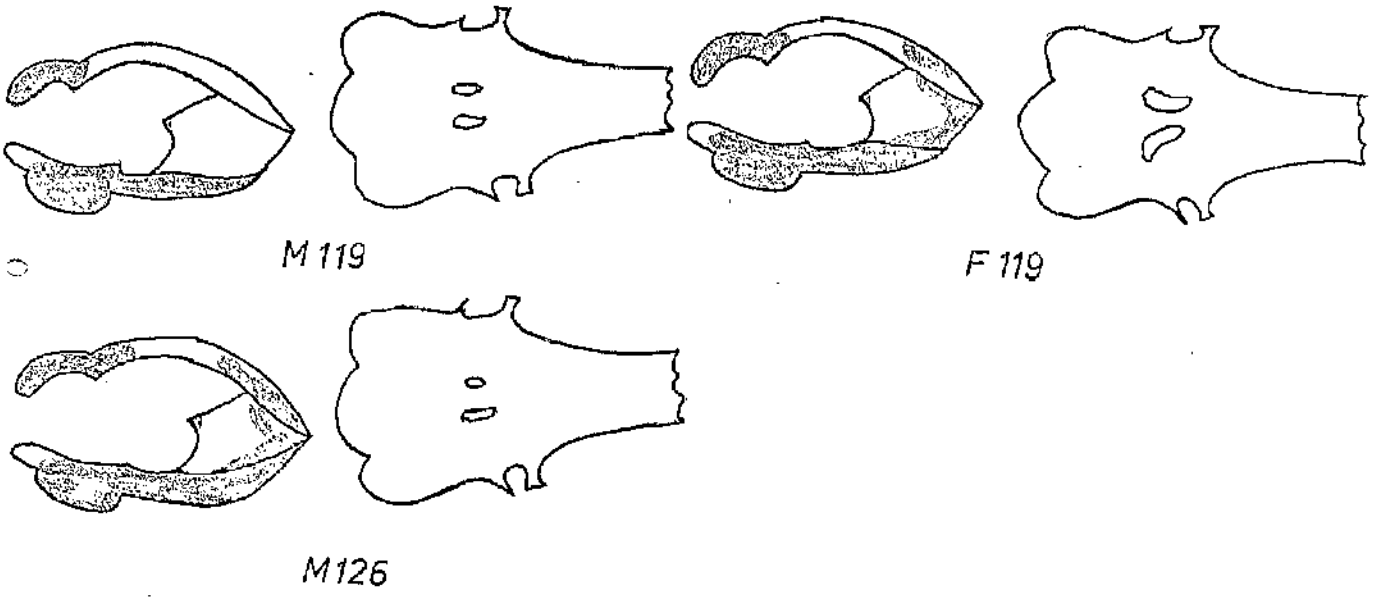


Fig. 118d

Figs. 118 a-d Extent of pneumatisation revealed by direct examination of sectioned surface of neurocranium compared to areas of translucence in White Leghorn birds of known ages

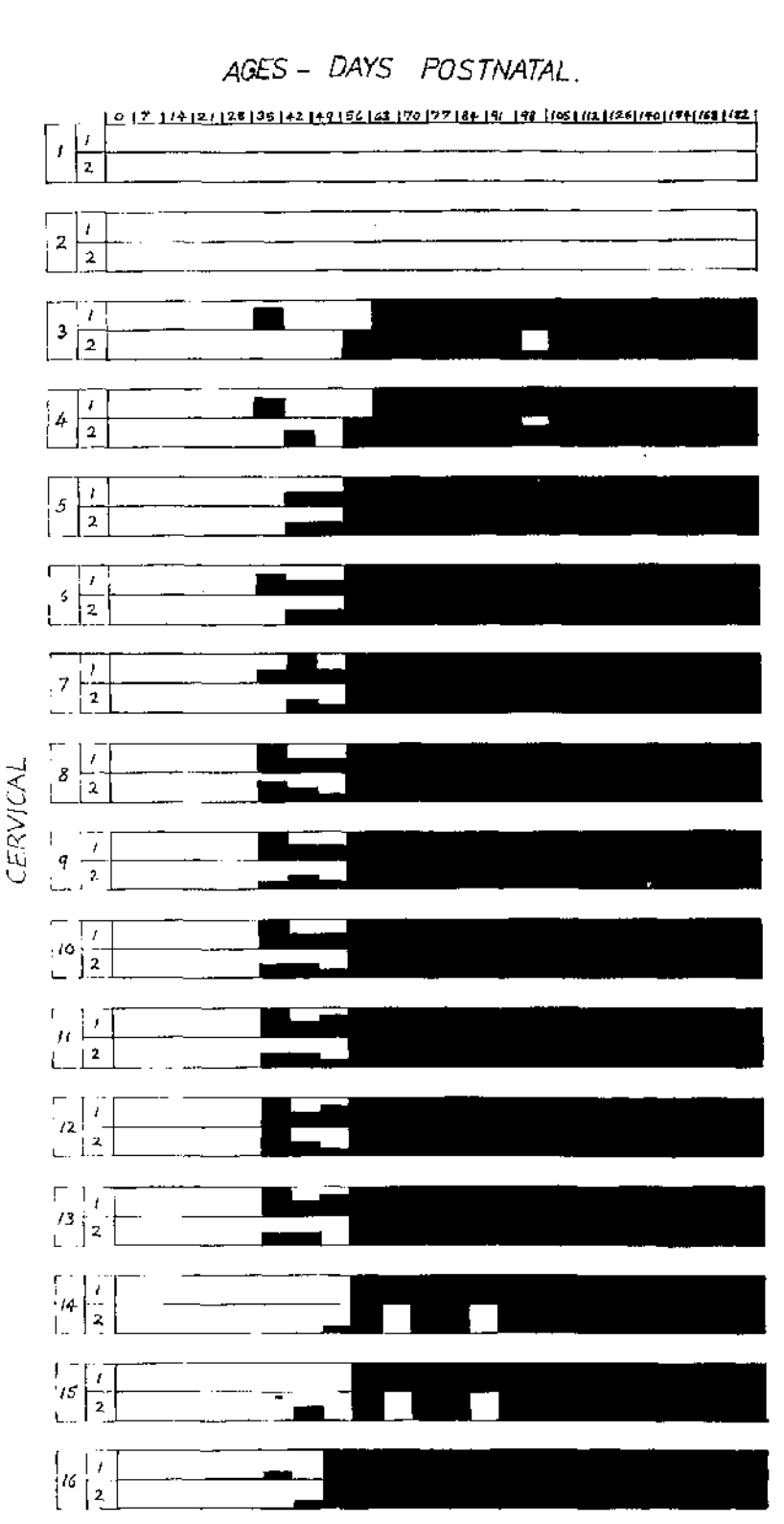


Fig. 119 Occurrence of pneumatisation in cervical vertebrae in pairs of growing Golden Comet birds of known ages.

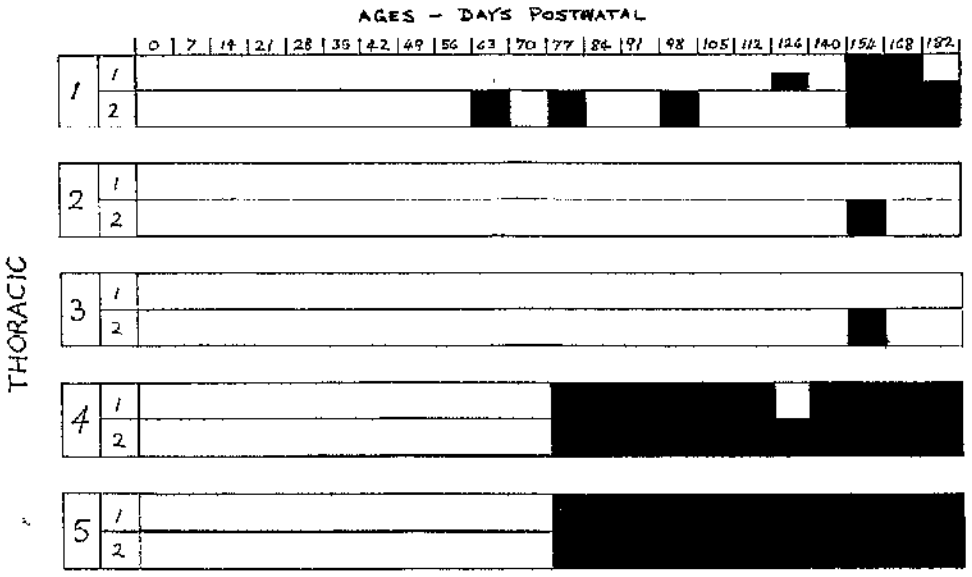


Fig. 120 Occurrence of pneumatisation in thoracic vertebrae in pairs of Golden Comet birds of known ages

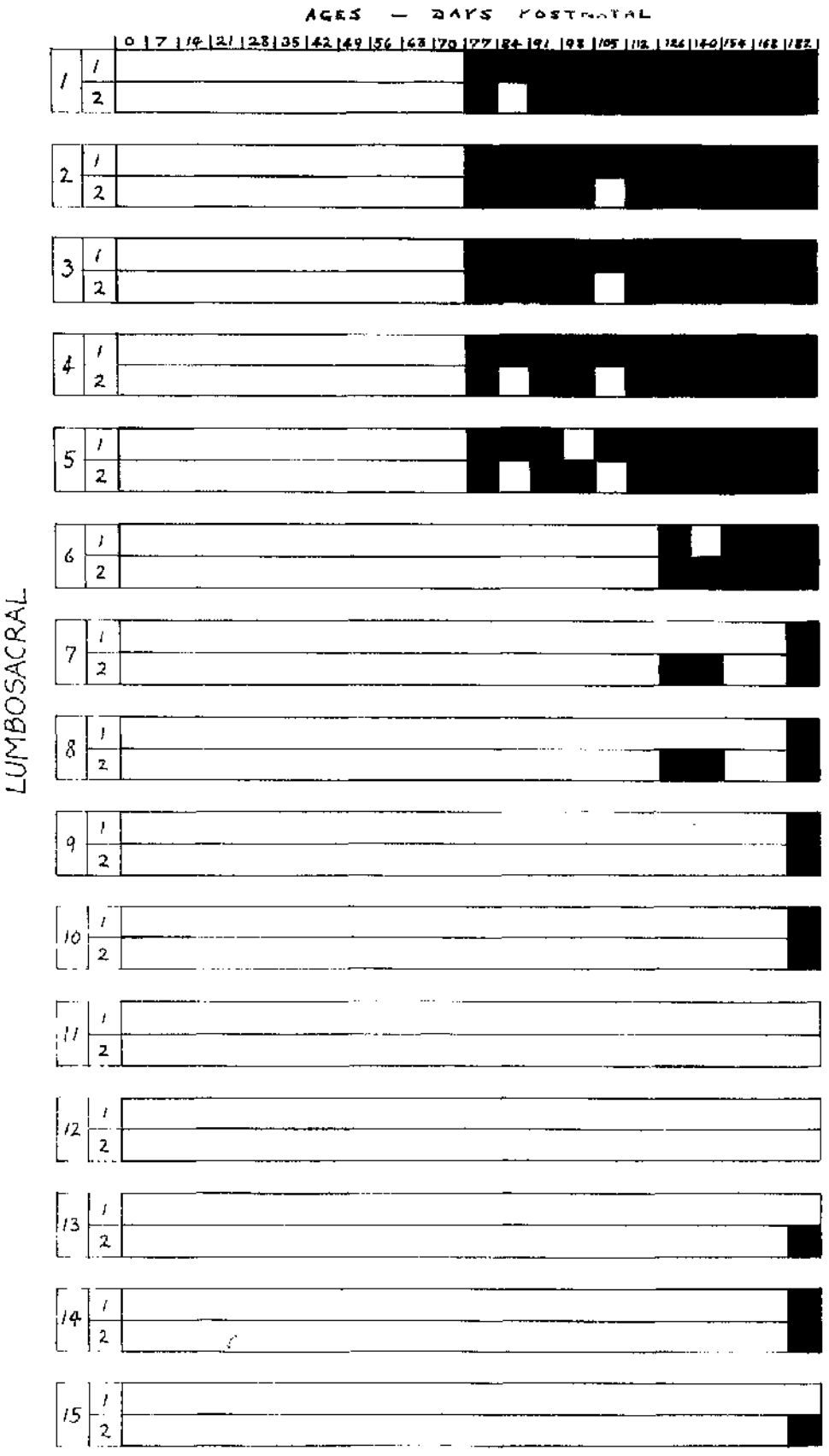


Fig. 121 Occurrence of pneumatisation in lumbosacral vertebrae in pairs of Golden Comet birds of known ages.

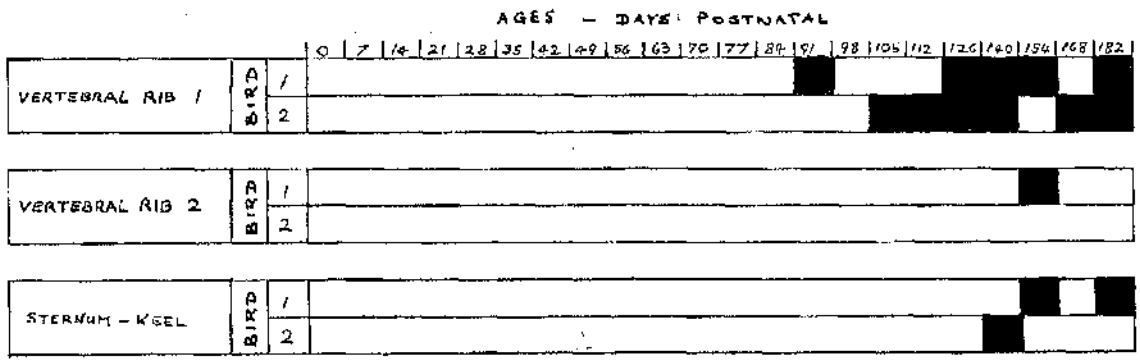


Fig. 122 Occurrence of pneumatisation in vertebral ribs and sternum in pairs of Golden Comet birds of known ages.

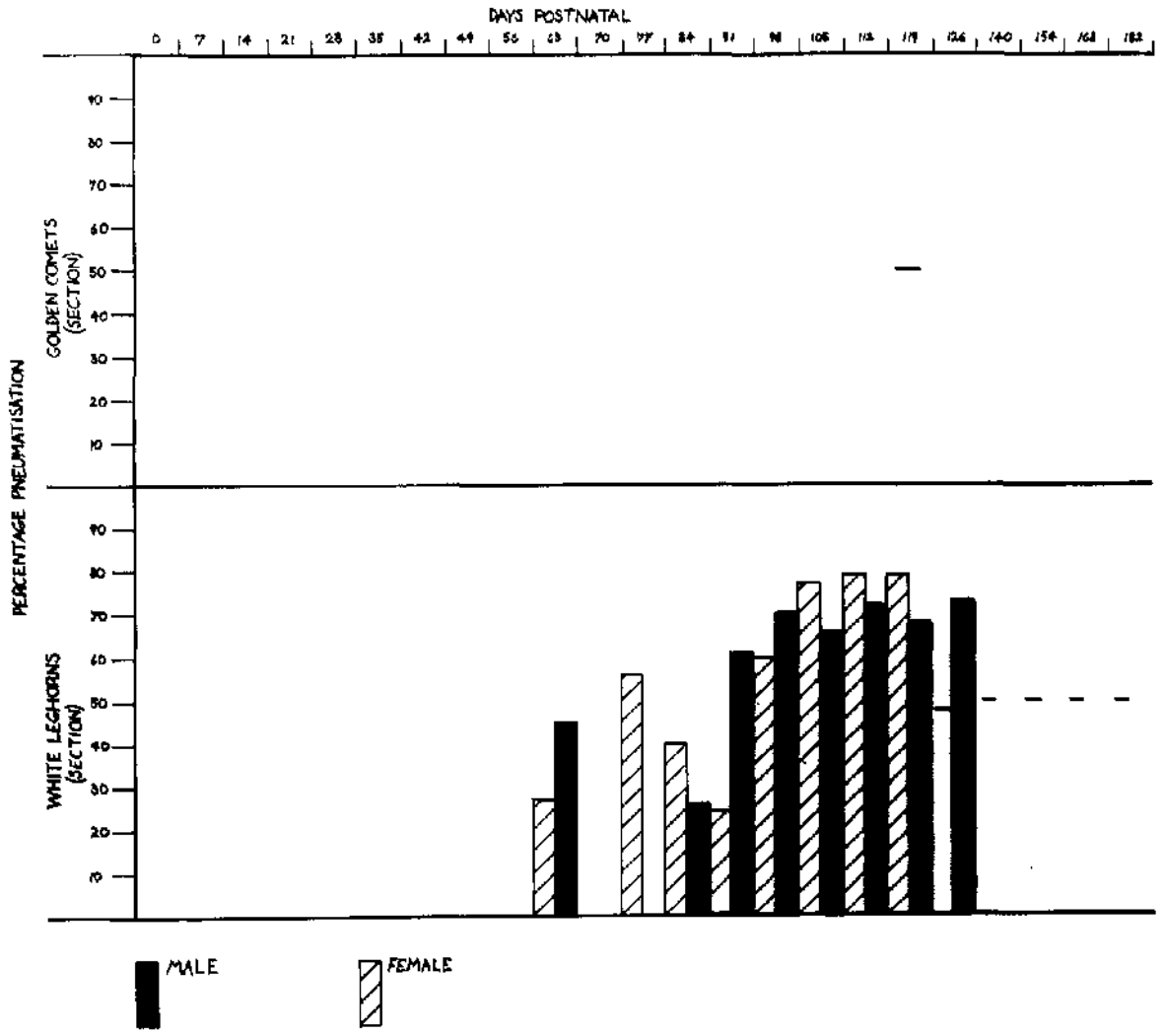


Fig. 123 Occurrence and extent of pneumatisation in left coracoid of birds of known ages.

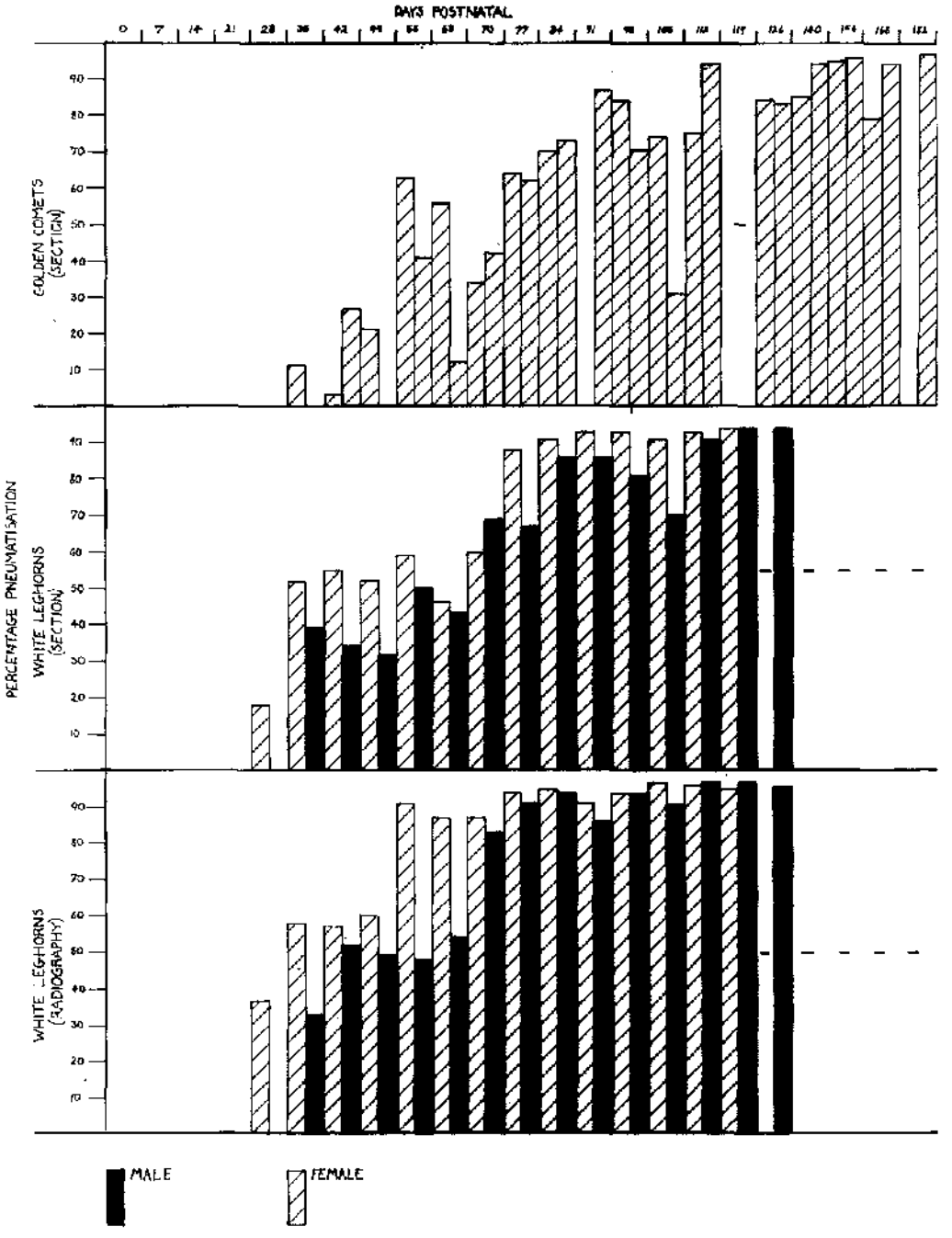


Fig. 124 Occurrence and extent of pneumatisation in left humerus of birds of known ages

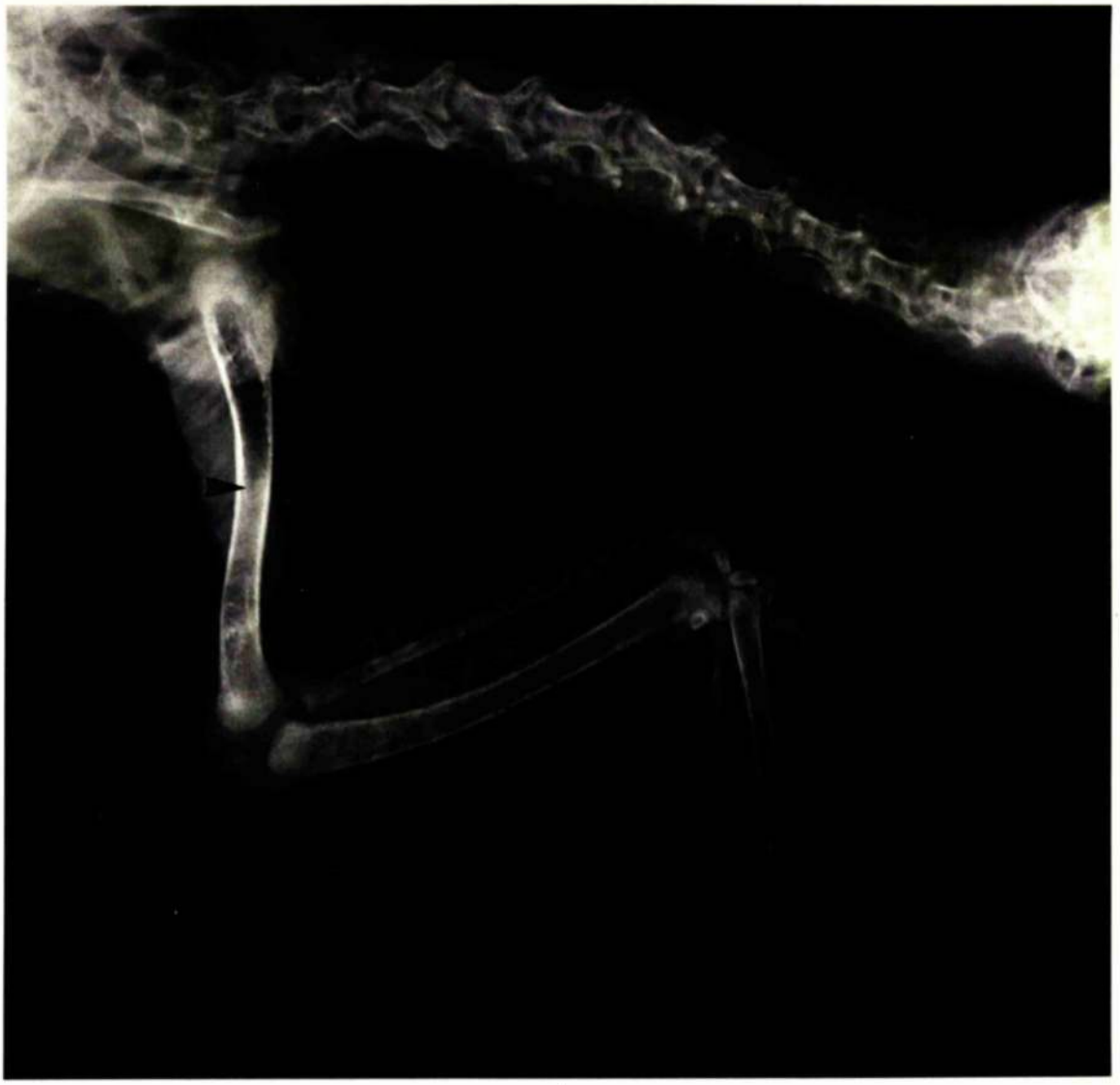


Fig. 125 Left wing 56 days (M56)
Lateral radiograph
arrow indicates distal limit of pneumatisation
of humerus